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Outlook in tissue-engineered magnetic systems and biomagnetic control

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Abstract

The advancement of tissue engineering strategies has opened up new therapeutic avenues in the regeneration of many musculoskeletal tissues and cell niches. The burst of research in nanotechnology associated with tissue engineering brings inputs for the precise control of cells and cellular environments, that can play an important role in the development of these new therapies.

Magnetic actuation, especially in combination with magnetic nanoparticles, may be a valuable tool in the interaction with living systems, such as stem cell guidance, retention, stimulation, and differentiation. Advances in the field of magnetic technology have also enabled the fabrication of increasingly complex systems such as cell sheets, organoids, or bioprinted scaffolds. Our Opinion article highlights this promising field of research and attempts to cover some of the most recent contributions to both tissue engineering and regenerative medicine.

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Introduction

Globally, the number of prevalent cases of musculoskeletal disorders was 1.3 billion in 2017 [1], representing a burden for patients and healthcare systems. Alternatives to conservative or surgical repairs aiming at restoring tissues to its primary functionality, are being pursued in tissue engineering (TE) and regenerative medicine (TERM) approaches. TE research has a growing impact in the development of strategies for controlling cells, cell environments, scaffolding systems, and 3D tissue models that resemble living tissue dynamics. Concomitantly, magnetic-based technologies bring additional and unique characteristics such as biofunctionalization, monitoring, and real-time control either in cellular-based approaches or biomaterial design. Consequently, the complex mimicking of living tissues and milieus demand integrative approaches to efficiently deliver therapeutic solutions.

Magnetically assisted actuation includes two main components, the magnetic nanoparticles (MNPs) and the magnetic field. Identifying and exploring the potential uses of MNPs and biomagnetic control will open vibrant paths for musculoskeletal tissue engineering and regenerative medicine strategies. The use of MNPs is promising because magnetic actuation can be operated at a distance in a remote and contactless way without direct interaction with the biological system. Therefore, magnetic-based nanoparticles technology can impact the biomedical field under controlled and precise actuation of magnetic field, being guided and concentrated at the desired site allowing real-time non-invasive treatment. Moreover, by allowing remote monitoring, they offer new perspectives either in diagnostics or therapeutics. MNPs are being used in biomedical theranostic approaches such as drug carriers [2], contrast agents in current use clinical imaging such as magnetic resonance imaging (MRI) [3], and are also clearly advancing the field of neurodegenerative disease and cancer diagnosis, monitoring, and therapy, especially due to its high multifunctionality [4]. Magnetic particles have also application in magnetic hyperthermia [5], in which the influence of the cell internalization on dynamic magnetic response of the particles has shown to depend on their size, intracellular viscosity, and aggregation state [6]. Other nanoparticles such as gold-based with theranostic properties can improve the detection of diseases [7,8], or incorporating graphene oxide (GO-MNPs) for functionalization with specific antibodies in signaling activation [9]. Notwithstanding, iron oxide-based MNPs are

probably the most widely studied magnetic materials and the most commonly used due to their biocompatibility, superparamagnetic behavior, low costs, and easy production.

Therefore, current studies investigate different designs of these MNPs only by partial substitution of iron by other metallic elements such as manganese, cobalt, nickel, or zinc [10], while maintaining their superparamagnetic behavior.

The research progress in iron oxide nanoparticle formulations has culminated in the FDA approval of different nanoparticles for clinical use, such as i) Feraheme® for iron deficiency, ii) Combidex® and Sinerem® as a magnetic resonance imaging (MRI) agents, iii) Nanotherm® for cancer treatment, iv) Lumirem® as an oral gastrointestinal tract imaging agent [11].

Pioneering studies over the past decade introduced tools capable of perturbing mechanosensitive cell receptors this way addressing signaling pathways implicated in development and homeostasis. Such tools include MNPs and magnetic force conditioning, which have been widely used to remotely activate mechanotransduction via different cellular receptors or ion channels [12–14]. MNPs attached to a specific receptor are magnetically induced to move creating pN forces sensed and transduced from the cell exterior to the cytosol promoting mechanotransduction mechanisms [15,16]. A recent example is that magnetic stretching provided to MNPs conjugated to specific integrin ligands activates AKT/mTOR pathway via integrin β1 to regulate collagen synthesis by human tendon cells [17].

From cells triggering to the fabrication of smart-responsive scaffolds, MNPs and biomagnetic control have enabled a multitude of applications for cellular stimulation either in 2D or 3D cultures. Recent progresses on biomaterial-guided cell behaviors have been exploring the incorporation of MNPs and/or the use of magnetic field in the fabrication of 3D scaffolds [18]. An example of the latest was reported by Parfenov and co-authors [19] resorting to magnetic levitation assembly of calcium phosphate particles for the purpose of bone tissue engineering. On the other hand, doping biomaterial matrices with MNPs permit the responsiveness of the structures within a magnetic field. As such, electrospun fiber conduits incorporating oleic acid-coated iron oxide nanoparticles provide surface nanotopography and guidance for neuron cells under manipulation of a magnetic field [20].

Notwithstanding, the translation of TE research into new regenerative therapies is still a grand challenge of this era, with a long way to go to translate magnetic TE from bench to bedside.

Magnetic application: a tool in tissue engineering

The modulation of inflammation is a key aspect in tissue repair in which magnetic actuation can play a role. In most cases, ideal strategies need a balanced regulation of the inflammation stage that is largely orchestrated by inflammatory cells [21]. A cell-material approach for controlling macrophages adhesion and subsequent polarization was developed by Kang et al. (Figure 1a), using a remote magnetically controlled nanocage conjugated to an RGD-coated gold nanoparticle [22]. The uncaging of RGD temporally encouraged macrophages adhesion and subsequent M2 phenotype while inhibiting their M1 phenotype, *in vitro* and *in vivo* [22]. A better understanding of how MNPs interact with immune cells *in vitro* and *in vivo* can therefore bring improvements in the delivery of immunomodulatory cues for successful therapies.

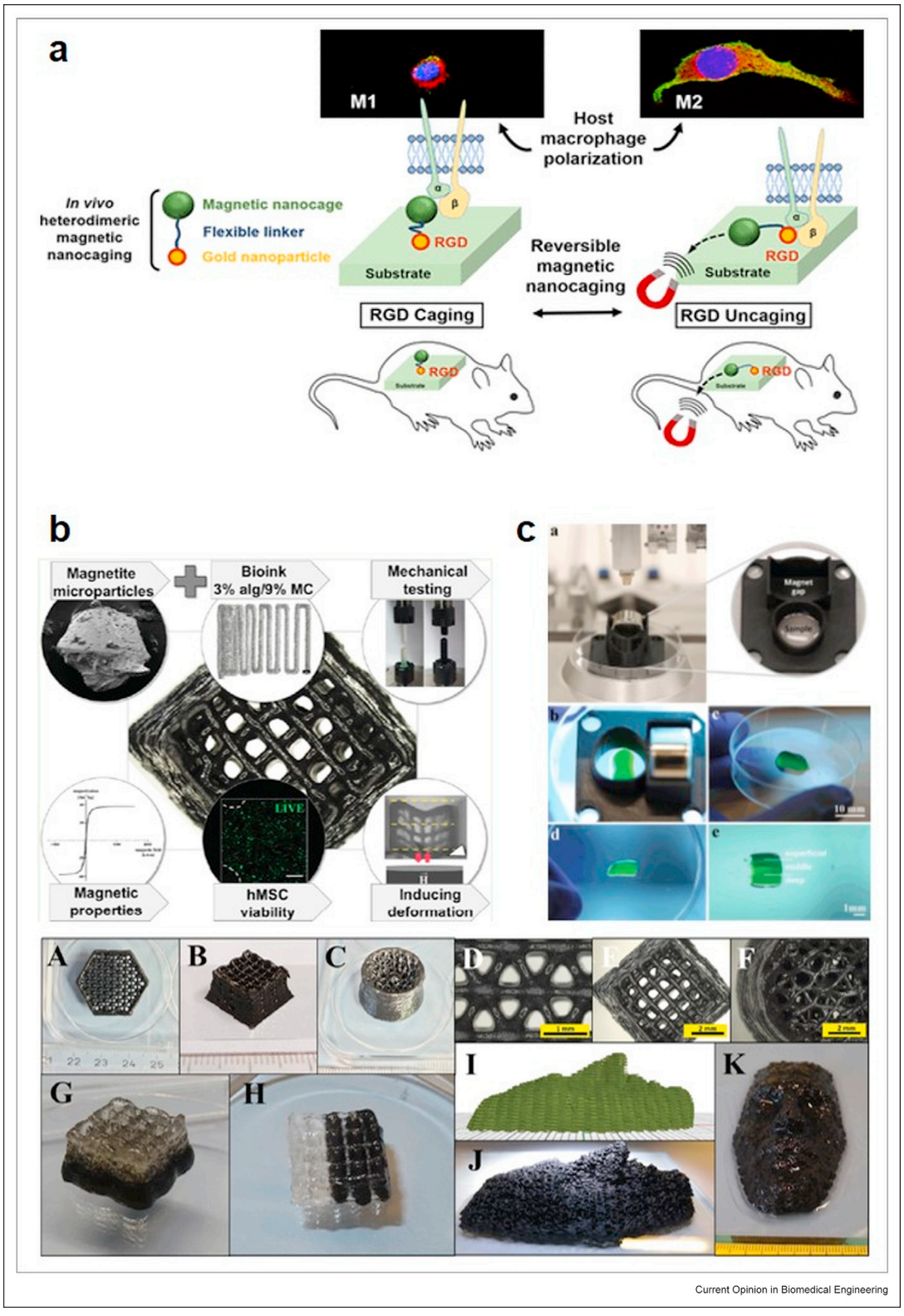
Bioprinting strategies have been used in the last few years to engineer 3D scaffolds through the precise deposition and assembly of materials and cells. Magnetic-assisted bioprinting technology is a new direction of bioprinting. An enticing study by Spangenberg et al. demonstrates the feasibility of bioprinting a magnetic bioink comprised of alginate, methylcellulose, magnetite microparticles, and human mesenchymal stem cells [23] (Figure 1b). Scaffolds proved to be stable and demonstrated high shape fidelity as well as viability of embedded cells. The cell-laden scaffolds were investigated under a cyclic magnetic field with a low frequency of 0.05–0.37 Hz, showing to be deformed in a reversible and tailored way [23]. With this work, the authors propose a magnetic system that enables spatial deformation and stimulation of encapsulated cells in a magnetic field.

Magnetically assisted bioprinting is being also used to promote fiber alignment, such as in a work by Betsch et al. in which it was used a custom-designed bioprinter incorporating a 2 mT magnet (Figure 1c) that forces real-time remodeling of the bioink while bioprinting [24]. Cell-free and cell-loaded hydrogels of agarose and type I collagen, with and without iron nanoparticles were bioprinted into a three-layered construct, with alternating layers of aligned and random fibers to resemble the structure of native articular cartilage. In this proof-of-concept study, authors demonstrated that human knee articular chondrocytes bioprinted constructs supported the production of a cartilaginous matrix after 21 days of culture [24].

Magnetically driven modulation of cell behavior in 2D or 3D

The concept of magnetic force-based tissue engineering (Mag-TE) makes use of MNPs and magnetic force in the construction of magnetic-responsive cell sheets. Cells are labeled with MNPs and forced into a cohesive tissue-like construct prompting cell–cell interactions and adhesion. This technology is being effectively used

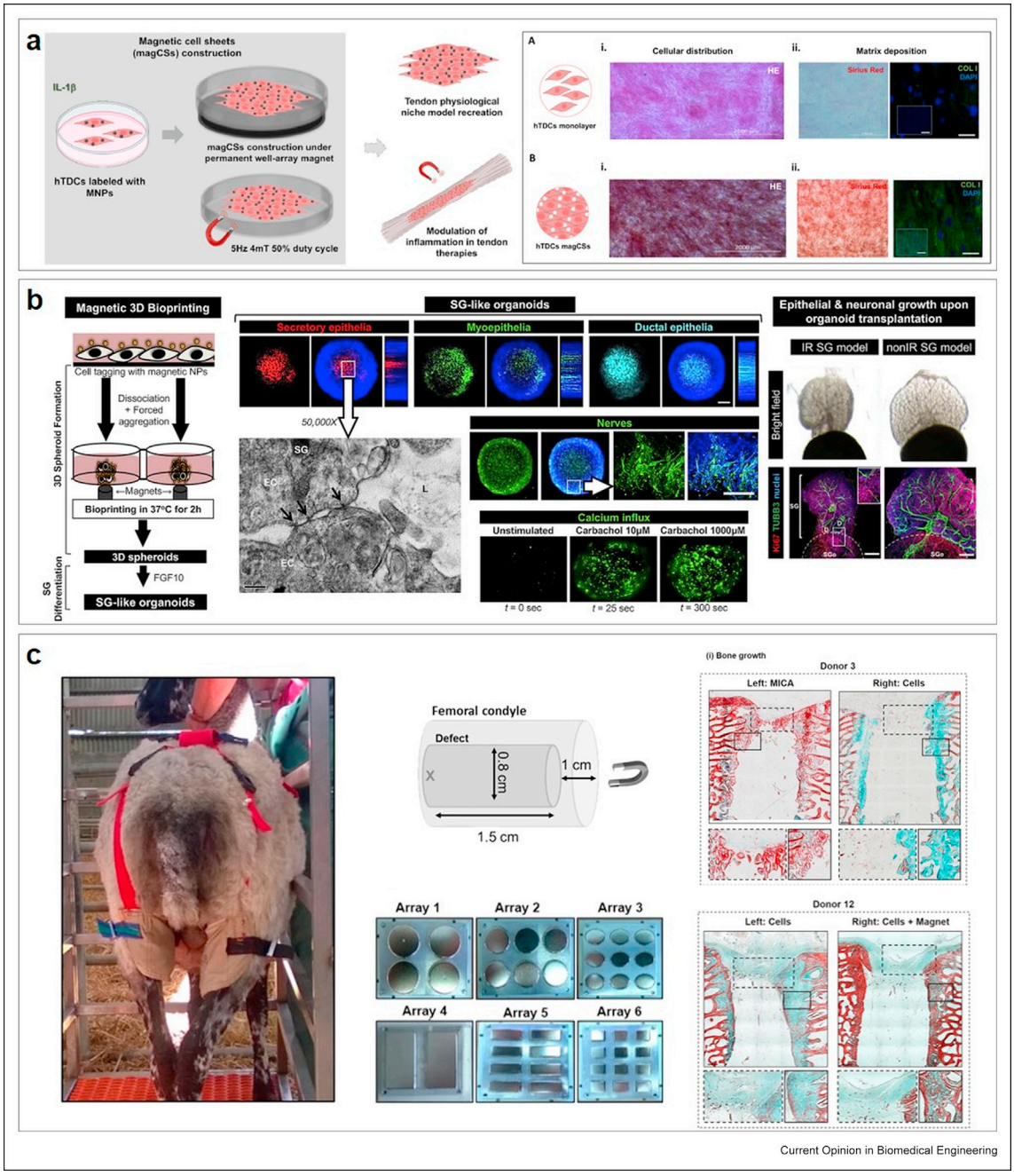
Figure 1



Remote magnetic control. **a**) Magnetic nanocage regulates the adhesion and polarization of macrophages both *in vitro* and *in vivo* (Reprinted with permission from Ref. [17]. Copyright 2018 American Chemical Society). **b**) 3D-plotted composite scaffolds comprising a magnetic bioink based on alginate (alg, 3%) and methylcellulose (MC, 9%) with incorporated magnetite microparticles (25% w/w) (Reprinted with permission from Ref. [18]. Copyright 2021 American Chemical Society). **c**) Magnetically assisted 3D printing system (4D bioprinting) applying magnetic force for real-time remodeling of the bioink while bioprinting (Reproduced with permission from Ref. [19]. Copyright 2018 Wiley-VCH).

in building scaffold-free cell constructs to be used in the regeneration of osteochondral [25], bone [26], and tendon [27,28] defects. In a recent study by Vinhas et al., cell sheets constructed from human tendon-derived cells were approached as a pathophysiological model of inflammation [28] (Figure 2a). In order to study inflammation activity and tendon cell responses in inflammatory environments that are associated with tendinopathic conditions, the cell sheets were exposed to IL-1β, a well-known pro-inflammatory cytokine.

Figure 2



Magnetically assisted actuation. **a**) Magnetic cell sheets (magCSs) constructed with human tendon-derived cells (hTDCs) and magnetic nanoparticles (MNPs) to study inflammation activity (Reprinted from Ref. [23], Copyright 2021, with permission from Elsevier). **b**) Magnetic 3D bioprinting to generate innervated secretory epithelial organoids from a neural crest-derived mesenchymal stem cells tagged with MNPs and spatially arranged with magnets (Reprinted from Ref. [24], Copyright 2018, with permission from Elsevier). **c**) Magnetic ion channel activation (MICA) technology translated to a pre-clinical ovine model (Reproduced with permission from Ref. [41]. Copyright 2018 Springer Nature).

Overall, the application of pulsed electromagnetic field (PEMF) showed a modulatory action over the inflammatory profile of IL-1β primed cell sheet constructions, via MAPK(ERK1/2) pathway [28]. In this work, the authors evidence the role of PEMF-assisted immunomodulatory effects in cell-based therapies and suggest the magnetic cell sheets as a scaffold-free patch for tendon regenerative therapies.

Envisioning structural resemblance to the corresponding tissue, organoid systems had emerged as models to investigate disease, drug screening, cell therapies, and personalized medicine. It was recently proposed an interesting magnetic three-dimensional bioprinting strategy that enables the fabrication of robust spheroids and the formation of innervated secretory epithelial organoids with different salivary gland (SG)-like cellular compartments [29]. This approach relies on tagging human dental pulp stem cells (hDPSC) with MNPs and assemble into spheroids using neodymium magnets (Figure 2b). MNPs-free culture system was used as control of the experiment. Then, salivary epithelial and neuronal differentiation stage started with specific culture media, of which FGF-10 and neurobasal medium with N2 were part, for up to 8 days. The organoids were then transplanted into excretory ductal area of developing SG *ex vivo* mouse model. As main results, the developed organoids stimulated epithelial growth in both irradiated-damaged SG and healthy glands [29]. This study highlights the fabrication of organoids with the incorporation of MNPs, a feature that may bring additional contactless control over spatial cell niche arrangements. Later on, in a similar study, the same group of authors used a magnetic three-dimensional levitation culture system to assemble and levitate magnetized primary SG-derived cells allowing them to produce their own extracellular matrices [30].

Magnetic responsiveness empowers the applicability of 3D biomaterials for TERM practices as easily actuable mechanostimulation platforms. In this matter, our group developed a tendon mimetic hierarchical magnetic fibrous scaffold that enabled remote magneto-mechanical stimulation of human adipose stem cells (hASCs), steering YAP/TAZ signaling pathway [31]. This stimulation showed a positive impact on the expression trends of anti-inflammatory/pro-healing and matrix remodeling genes and increased expression of tendon-related markers in comparison to non-stimulated conditions [31]. Interestingly, we lately described the combination of a fibrous aligned superparamagnetic scaffold and targeted Activin A type II receptor (ActRIIA) in hASCs that showed improved mechanotransduction response towards tenogenic commitment, via ActRIIA/Smad2/3 cascade [32]. In this work, an alternating magnetic field was used to promote external triggering over TGF-β/Smad2/3 signal transduction, by means of functionalized MNPs-ActRIIA. An exciting feature of magnetic systems and magnetic therapy devices is the ability to remotely control stem cell behavior, demonstrating the potential of actuable constructs for translational studies.

3D-printed titanium scaffolds with a magnetic coating containing Fe_3O_4 nanoparticles and polydopamine were developed by Huang and co-workers [33,34] for bone applications. The composite scaffolds showed increased osteogenic differentiation of hBMSCs *in vitro*, which was associated with the TGF-β-Smads pathway, confirmed by proteomic evaluation analysis. *In vivo* implantation in femoral epicondylar defects in rabbits showed higher amounts of new mineralized tissue and the volume of newly formed bone was significantly higher in magnetically stimulated scaffolds after 12 weeks [34].

In a study by Wang et al., magnetic lanthanum-doped hydroxyapatite/chitosan scaffolds were claimed to recruit endogenous stem cells and promote host-to-scaffold immune response for bone regeneration [35]. As detected by flow cytometry for CD206, magnetic scaffolds could promote seeded macrophages to polarize toward the M2 phenotype and promote osteoinductive phenotype of rBMSCs shown by ALP activity and bone-related genes expression. Moreover, histomorphometric values of rat calvarial explants after 12 weeks were higher in magnetic scaffolds than in non-magnetic scaffold groups. The authors attribute the greater osteoconductivity and stem cell recruitment effects to magnetic $SrFe_{12}O_{19}$ nanoparticles incorporated in the scaffolds [35].

Magnetic-responsive hydrogels have also attracted intensive researches in tissue engineering that were very recently reviewed [36,37]. Beyond controllable behavior features, the incorporation of MNPs grants the possibility of creating hydrogels with anisotropic microstructure. An example is the magnetically responsive nanocomposite hydrogel composed of collagen type I and aligned iron oxide nanoparticles developed by Xu et al. for tendon applications [38], or the anisotropic alginate hydrogel composed of magnetic short fibers for neural tissues regeneration fabricated by Ghaderinejad and co-workers [39]. Recent reports of magnetic hydrogels with good biomechanical stability have been also proposed for cartilage [40,41], muscle [42], and bone [43].

Translational challenges

The active control of stem cell responses remains a challenge once implanted in the body. Some pre-clinical studies are investigating imaging, mapping, and actuation of stem cells, in which the contribution of MNPs gains significant contribution in regenerative therapies. A comprehensive overview of functionalized MNPs in pre-clinical and clinical biomedical applications is given by Dash et al. [44]. A magnetic nanoparticle-based

Table 1

Recruiting studies using magnetic therapy. Source: ClinicalTrials.gov.

ClinicalTrials.gov Identifier	Title	Condition	Treatment
NCT04109638	Pulsed Electromagnetic Field (PEMF) Therapy for Post-operative Pain Following Orthopedic Surgery	Knee Injuries Shoulder Injuries Pain, Postoperative	Device: Endonovo SofPulse
NCT04859842	Comparison of Two Electrotherapy Methods on Chronic Low Back Pain	Low Back Pain	Pulse Electromagnetic Field
NCT04106986	The Effect Of Pulsed Electromagnetic Field And Progressive Resistance Exercise On Knee Osteoarthritis	Knee Osteoarthritis	Pulsed electromagnetic field and Progressive resistance exercise
NCT04255407	Effect of Biophysical Stimulation on Intraspongious Bone Edema in Anterior Cruciate Ligament Reconstruction	ACL Injury Pain	Device: I-ONE® therapy Drug: Anti-inflammatory drug

delivery of oligonucleotides in the central nervous system of rats was investigated for treating brain diseases such as Parkinson's [45]. In this work, magnetofection was employed for the delivery of the polymeric magnetic particles NeuroMag®complexed with miR-134 inhibitors, in a specific region of the brain. The magnetic complexes reached neurons and glial cells, efficiently decreasing miR-134 content compared to controls [45].

Magneto-controlled delivery systems comprising the use of MNPs and magnetofection have proven to be a possible strategy *in vivo* [45–47] that can be further examined in pre-clinical models of several disorders.

Magnetic resonance imaging (MRI) was used to monitor MNPs labeled MSCs biodistribution in the knee joints of an ovine osteochondral injury model [48,49]. By means of MRI tracking, the signal of MNPs could be detected within the synovium instead of MSCs 'honing' to the defect [48,49]. Moreover, in a pre-clinical example of a translational biomagnetic approach successfully achieved in an ovine model of bone injury (Figure 2c), it was possible to trigger and control stem cells remotely through mechanical forces [50]. A STRO-4 positive population of oMSCs was labeled with MNPs, previously functionalized with TREK-1 antibody, encapsulated in an ECM gel, and implanted within a femoral defect. The authors use MNPs to remotely deliver mechanical stimuli to TREK-1 receptor, activating cells signaling post-implantation. This was achieved via an external magnetic array, compatible with the ovine model, for magnetic ion channel activation (MICA). After 13 weeks, micro-CT and histological evaluation showed that MICA-treated defects repaired to a greater degree in comparison to the controls [50].

In spite of the promising effects on musculoskeletal tissue engineering and regenerative medicine demonstrated by magnetically assisted technologies, as previously discussed, up to date, there are very few clinical trials being performed. The database ClinicalTrials.gov was searched for currently recruiting participants' studies, using the keywords "magnetic" associated with "musculoskeletal disorders". The retrieved trials are summarized in Table 1, focusing on the application of magnetic therapy, mostly pulsed electromagnetic field, for the treatment of different musculoskeletal injuries.

Conclusions

Globally prevalent musculoskeletal disorders require new therapeutic solutions to repair damaged or diseased tissue. In this review, we have highlighted the enormous potential of magnetically assisted technologies being currently used in TERM approaches.

When it comes to TERM, MNPs and magnetic technology bring promising breakthroughs in promoting important cellular events and at increasing levels of structural and functional complexity in tissue engineered models. Magnetized cells and the application of magnetic fields can be an interesting engineering approach in the construction of *in vitro* miniaturized model systems of organs, promoting stem cell aggregation and retention, towards more physiologically relevant spatial organization. Furthermore, smartly instructive systems combining diagnostic and therapeutic features are being chased for the progression of TERM. Both magnetic materials and biomagnetic control have potential as clinically translatable approaches, together or separately, extending into the regeneration of different tissues and cell niches. Moreover, there is a growing interest in the control of the immune response in regenerative medicine approaches. Thereafter, bringing immunology and TE together might improve biomaterial developments for tissue regeneration, in which magnetic technologies will definitely have an enthusiastic role.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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