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Skeletal muscle tissue engineering using magnetic nanoparticles: a comprehensive review

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Skeletal muscle tissue engineering (SMTE) is a promising tissue therapy that can innovatively address the challenges arising from trauma, diseases, or congenital disabilities. Advances in bioengineering have established multifaceted paradigms for SMTE, wherein magnetic nanoparticles (MNPs) have emerged as dynamic bioactive therapeutic agents. Owing to their magnetic properties, versatile surface modification, and interactions with biological systems, MNPs show significant promise when combined with cell and tissue therapies. This review aims to study the importance of MNPs in SMTE with emphasis on scaffold transplantation, bioactive agent delivery, myogenic differentiation, and remote mechanical stimulation. It highlights the predominant types of MNPs employed, including iron oxide-based nanomaterials and their fabrication, modification, and biocompatibility. Moreover, steady progress in the area of scaffolding, such as electromagnetic-driven scaffolding, advanced magneto-mechanical stimulation techniques, and the synergistic application of MNPs with other smart materials, is emphasised. This review underscores issues related to dosage, cytotoxicity, and *in vivo* translation and proposes further research steps to fully harness the therapeutic potential of MNPs for skeletal muscle functional regeneration. This paves the way for the engineering of smart, magnetoresponsive systems for clinically viable muscle repair strategies.

1. Introduction

Skeletal muscle is formed by a highly structured array of aligned muscle fibres in an ordered hierarchy of layers of the extracellular matrix (ECM). Under healthy conditions, the tissue's structure and function are restored following the regenerative process.[1,2] Subsequent to injury, macrophages initiate the repair process by removing necrotic muscle fibers and degraded extracellular matrix (ECM), thereby restoring tissue homeostasis.[3] This is followed by activation of resident muscle stem cells (satellite cells, SCs), which proliferate and differentiate to drive muscle regeneration.[4] However, in cases of severe damage (*i.e.*, major trauma), severe tumour growth, and extended degeneration (*e.g.*, congenital myopathies),[1] substantial loss of muscle tissue is apparent, necessitating surgical repair. However, autologous skeletal muscle replacement is associated with substantial limitations, including donor-site morbidity and difficulties in achieving adequate tissue volume, vascularization, innervation, and long-term functional integration. Therefore, alternative approaches have been proposed, including the use of skeletal muscle tissue engineering as a therapeutic modality for volumetric muscle loss (VML).[5,6]

Such an approach relies on the ability of myogenic stem cells to proliferate and differentiate, which underlie their regenerative abilities and are essential for muscle growth.[7] These stem cells can be derived from multiple sources including primary sources (*i.e.*, the progeny of satellite cells), induced pluripotent stem cells (iPSCs), or other stem cell sources (*e.g.*, mesenchymal stem cells (MSCs)). Cell therapy has been explored, in which stem cells can be directly injected into the defect site to enable *in vivo* differentiation. However, this approach has several limitations including limited integration and a lack of a structural scaffold (ECM) to support regeneration. Skeletal muscle injuries caused by trauma, surgical procedures, and degenerative diseases present significant clinical challenges due to the limited regenerative capacity of muscle tissue. While mild injuries can often heal through endogenous muscle repair mechanisms, severe muscle loss, as seen in conditions such as volumetric muscle loss (VML), requires advanced therapeutic

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interventions. Traditional treatment approaches, including surgical grafting and physiotherapy, often fail to restore complete function, necessitating the development of novel regenerative strategies.

Therefore, *in vitro* tissue engineering of skeletal muscle (TESkM) provides an opportunity to support myogenic differentiation within a three-dimensional (3D) matrix to recreate the skeletal muscle structure before implantation.[8,9] Tissue engineering has emerged as a promising solution for muscle regeneration by combining biomaterial scaffolds, cellular therapies, and bioactive molecules. Within this field, magnetic nanoparticles (MNPs) have gained significant attention due to their unique physicochemical properties including biocompatibility, magnetic responsiveness, and their ability to influence cell behaviour. These nanoparticles, primarily composed of iron oxide (Fe_3O_4 or γ-Fe_2O_3), can be incorporated into scaffolds or used as carriers for bioactive molecules, enhancing muscle tissue repair through controlled cell alignment, targeted drug delivery, and magnetic field-mediated stimulation. Several suitable hydrogel-based matrices for cultivating myogenic cells have demonstrated the successful development and function of biomimetic skeletal muscle.

However, to achieve optimal development and maturation of TESkM, electrical and mechanical stimuli must be applied to mimic native physiological signals. Despite progress towards the maturation of functional TESkM and evidence supporting functional regeneration in preclinical models, several issues prevent successful clinical translation of TESkM into human tissues. The greatest challenge is in producing sufficient volume (and mass) of TESkM to support functional regeneration in situations of VML, where significant mass has been lost. To achieve this, it is necessary to scale up the production of myogenic cells (and other required cell populations – endothelial cells, fibroblasts, neurons, *etc.*) and scaffold biomaterials to meet the requirements for full replacement.[9,10] Upon implantation, full neovascularisation and perfusion of the tissue are required for transporting waste products, nutrients, carbon dioxide, and oxygen effectively. Furthermore, adequate reinnervation is necessary to support regeneration and functional muscle recovery.

Hence, surgical expertise to transplant the muscle scaffolds into the damaged site is required.[10,11] Indeed, considerations of timing and rehabilitation, amongst other factors, will need to be evaluated for the TESkM graft to be successful.

To support the successful translation of TESkM for VML conditions, further advancements are required to overcome the above-outlined limitations. One such emerging field is nanotechnology, which has emerged at the forefront of rapid advances in novel therapeutic and diagnostic innovations across multiple fields of medicine.[12–14] One particular innovation of relevance is the development of magnetic nanoparticles (MNPs). MNPs exhibit unique magnetic properties due to their nanoscale size, typically 1–100 nm.[3] The magnetic characteristics can be affected by a variety of formulation and preparation techniques that are intended to achieve homogeneity in size, shape, and composition (*e.g.*, different salt combinations, ionic charge ratio, pH, and ionic strength).[15,16] Another important factor that controls the physical properties of MNPs is surface chemistry, which can be used to reduce potential toxicity and uptake in biological systems.[17] Additionally, MNPs can be readily "functionalized" with bioactive substances, such as peptides or drug molecules, to create unique engineering systems that can cross the cell membranes and exert pharmacological effects specific to a target tissue.[18] They have emerged as a significant innovation in tissue engineering, particularly for bone regeneration.[4,19] Their unique properties enhance the effectiveness of various therapeutic strategies and biomaterials used in tissue repair.[1] Magnetic particles, classified by their size, shape, and magnetic properties, exhibit unique physical and chemical properties that are essential for their applications in biomedicine and tissue engineering.[2] Their small size results in a high surface-area-to-volume ratio, significantly enhancing their reactivity and interactions with biological systems.

Magnetic nanotechnology approaches could help overcome the aforementioned constraints of SMTE by producing composites with features that closely resemble natural *in vivo* features. In tissue engineering, MNPs can enable specific features such as scaffold modification, cell labelling, or drug delivery within the chosen system. MNPs have recently been used to regulate cell activity. Thus, they can be used in a wide range of skeletal tissue engineering applications due to their intrinsic features. Furthermore, the intracellular delivery of MNPs results in cell clusters and enables the proper application of magnetic fields to align cells. In addition, they can be used to create more intricate tissue structures, as their functionalization can modulate cell signalling pathways.[20] The fundamental idea is to use MNPs under the influence of a magnetic field, even though they were not designed for this.

This review comprehensively explores the current applications of MNPs in skeletal muscle tissue engineering, covering *in vitro* and *in vivo* studies and ongoing clinical research. We discuss the benefits and limitations of using MNPs in muscle regeneration, highlight the key findings from preclinical studies, and examine the challenges associated with translating these promising approaches into applications in humans.

2. Magnetic nanoparticles: properties and types

MNPs exhibit superparamagnetic behaviour at the nanoscale, implying that they can be magnetised in the presence of an external magnetic field but lose magnetisation when the field is removed. Depending on the type of MNPs, they offer distinct advantages for various tissue engineering applications, enhancing the therapeutic efficacy, imaging capabilities, and overall biocompatibility (Fig. 1). MNPs can be directed to specific skeletal muscle tissue sites using external magnetic fields for targeted drug delivery,[11] improving therapeutic outcomes while minimising systemic side effects. MNPs can also be made with functionalised core–shell structures designed to release bioactive molecules in response to environmental triggers (*e.g.*, pH and

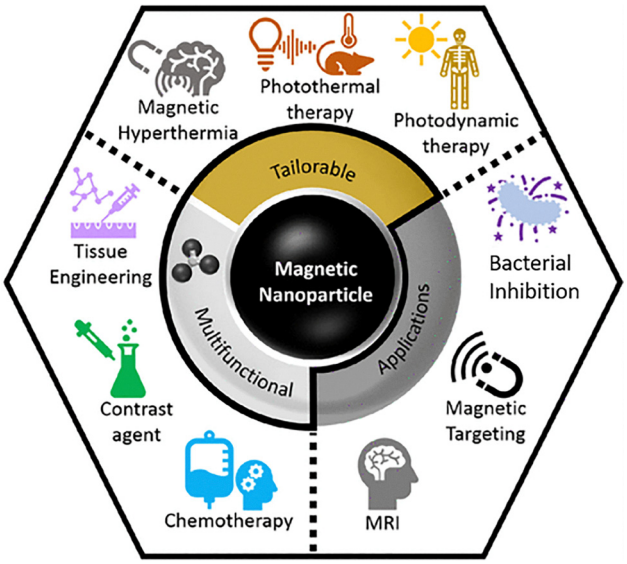

Fig. 1 Magnetic nanoparticles and their properties and types in biomedical applications.[22]

temperature), thereby enhancing localised treatment. There are a range of MNPs available; however, iron oxide nanoparticles are generally biocompatible and have been shown to promote cell adhesion and proliferation, which are crucial for the integration of engineered tissues with host tissues.[12,21]

The incorporation of MNPs into scaffolds can improve the mechanical strength and elasticity of the materials, mimicking the properties of native muscle tissue for load-bearing applications that require it to withstand physical forces.[13] The ratio of magnetic particles to polymers can be adjusted to achieve the desired stiffness and flexibility, enabling the optimisation of scaffolds for muscle applications. The application of magnetic fields can stimulate muscle stem cells and promote their differentiation into myotubes, aiding in the regeneration of damaged tissues. Reported mechanisms include the controlled release of growth factors supporting muscle cell proliferation and differentiation.[14]

Iron oxide nanoparticles can also serve as effective MRI contrast agents, enabling non-invasive monitoring of muscle tissue development and integration over time.[15] The magnetic properties of MNPs enable tracking of implanted materials or cells within muscle tissue, providing insights into their behaviour that would otherwise be impossible. Furthermore, incorporating conductive materials with magnetic nanoparticles can enhance the electrical conductivity of scaffolds, contributing to proliferation, differentiation, and functional regeneration. The key properties of the synthesized materials and the advantages they offer are listed in Table 1.

3. Cell types, growth factors, and their manipulation in tissue constructs

Myogenesis specifically refers to the process of muscle formation, particularly the differentiation of myoblasts into myotubes and subsequently into mature muscle fibres. This process is critical for muscle development and repair. It involves a series of tightly regulated stages characterised by the expression of key myogenic regulatory factors (MRFs) such as MyoD, Myf5, and myogenin. This process is essential not only during embryonic development but also for muscle regeneration following injury, highlighting its importance in tissue engineering and regenerative medicine.[23] These processes can also be recapitulated *in vitro* to support the development of TESkM.

As previously described, several cell types are required for successful skeletal muscle tissue engineering. For the myogenic cell component, mesenchymal stem cells (MSCs), primary muscle-derived cells (MDCs, progeny of satellite cells), embryonic stem cells (ESCs), and induced pluripotent stem cells (iPSCs) are available cell sources promoting myogenic differentiation and regeneration.

MSCs, derived from sources such as bone marrow and adipose tissue, can differentiate into myoblasts when exposed to specific growth factors, such as insulin-like growth factor 1 (IGF-1), and myogenic regulatory factors (MRFs), such as MyoD and myogenin. ESCs and iPSCs can also differentiate into myogenic progenitor cells under the influence of factors such as bone morphogenetic proteins (BMPs) and Pax7, enabling the regeneration of muscle tissue.

Using MNPs conjugated to antibodies, such as CD56/Neural Cell Adhesion Molecule (NCAM, a marker for muscle satellite cells and their progeny), provides a powerful strategy for controlling cell positioning within a tissue-engineered environment. This approach, often referred to as magnetic cell targeting, enables precise spatial control of cells by applying an external magnetic field. By attaching antibodies that bind specific cell-surface markers, researchers can facilitate the selective capture and retention of target cells at desired locations within scaffolds or hydrogels. This technique not only enhances cell organisation and interactions but also supports more effective myogenesis by ensuring that cells are positioned optimally for differentiation and integration into the surrounding tissue. Ultimately, this control over cell placement can lead to improved functionality and structural integrity in engineered muscle tissues. Magnetic fields can also positively influence myoblast proliferation and differentiation[24] by enhancing cell growth and promoting the expression of myogenic regulatory factors (MRFs) such as MyoD and myogenin. Magnetic fields also facilitate the alignment and fusion of myoblasts into multinucleated myotubes, essential for muscle fibre formation. Additionally, magnetic fields stimulate mechanotransduction pathways, enhancing interactions with the extracellular matrix and promoting the formation of more mature myotubes with better contractile properties. However, alterations in cellular architecture, extracellular matrix organization, the mechanosensing machinery, or associated downstream signaling pathways disrupt normal mechanotransduction and contribute to disease onset and progression. Mutations in proteins that normalise intracellular calcium signaling or in crucial components of the Rho and mitogen-activated protein kinase (MAPK) pathways can damage mechanotransductive signaling and

Table 1 Brief descriptions, synthesis methods, and key properties of different types of magnetic nanoparticles

Type of magnetic nanoparticles	Composition/ description	Typical synthesis routes	Key advantages	Physicochemical properties	Stability considerations (physiological media)	Degradation/ ion release behavior	Toxicity considerations
Iron oxide nanoparticles[5,6]	Magnetite (Fe_3O_4) – inverse spinel structure, high magnetic susceptibility; maghemite (γ-Fe_2O_3) – oxidized form with improved chemical stability	Co-precipitation, sol–gel synthesis, hydro-/solvothermal methods, thermal decomposition	High biocompatibility, chemical stability, ease of surface functionalization, scalable synthesis	Superparamagnetic behavior at the nanoscale, high saturation magnetization, low coercivity, and colloidal stability in aqueous media	Aggregation possible without steric/ electrostatic stabilization; surface oxidation ($Fe^{2+} \rightarrow Fe^{3+}$); zeta potential dependent dispersion stability	Biodegradable *via* lysosomal dissolution; Fe ions released under acidic pH; metabolized through iron homeostasis pathways	Generally low toxicity; excessive Fe^{2+} may induce ROS *via* the Fenton reaction; dose-dependent oxidative stress and inflammation
Magnetic nanocomposites[7,8]	Magnetic nanoparticles embedded within polymeric, lipidic, or inorganic matrices (*e.g.*, PVA, PLA, and chitosan)	*In situ* polymerization, emulsion/solvent evaporation, electrospinning, layer-by-layer assembly	Tunable mechanical and degradation properties, controlled drug release, enhanced cellular interactions	Tailorable magnetic response, improved dispersion stability, biodegradable, and bioresponsive behavior	Matrix prevents nanoparticle aggregation; enhanced steric stabilization; stability depends on polymer degradation kinetics	Controlled degradation of polymer matrix; gradual nanoparticle exposure; reduced burst ion release	Reduced acute cytotoxicity compared to bare MNPs; toxicity influenced by polymer type, degradation byproducts, and residual solvents
Core–shell magnetic nanoparticles[9,10]	Magnetic core (typically Fe_3O_4 or γ-Fe_2O_3) coated with silica, polymers (*e.g.*, PEG), or lipid shells	Stöber silica coating, surface-initiated polymerization, self-assembly, microemulsion methods	Reduced cytotoxicity, enhanced colloidal stability, site-specific functionalization, improved *in vivo* circulation	Core-dominated magnetic response with shell-controlled surface chemistry, reduced aggregation, high functional group density	High colloidal stability due to steric/electrostatic repulsion; shell thickness influences long-term stability; oxidation resistance	Shell slows ion leaching; degradation governed by shell composition (silica dissolution or polymer hydrolysis)	Lower ROS generation than bare cores; toxicity depends on shell material; PEG reduces immune recognition; silica shells may induce dose-dependent inflammatory responses

disturb cellular homeostasis.[25] Insulin-like growth factor-1 (IGF-1) functions as an autocrine hormone in muscle tissue, supporting muscle growth and regeneration. Direct mechanical stimulation of muscle cells can activate the AKT/mammalian target of the rapamycin (mTOR) pathway independently of paracrine or systemic signals from other cell types, promoting muscle growth.[26] Moreover, the transcription factor nuclear receptor subfamily 4 group A member 3 (NR4A3) regulates gene expression programs involved in muscle remodeling and metabolism.[27] Overall, this approach can enhance the regenerative potential of muscle tissue in tissue-engineering applications. MNPs enhance the mechanical properties of scaffolds such as gelatin, collagen, and decellularised extracellular matrix (ECM)[28,29] by increasing the tensile strength and stiffness, improving elasticity and flexibility, and promoting better cytocompatibility.

3.1 Magnetically controlled tissue alignment and directional growth

Magneto-active constructs established a three-dimensional culture milieu that directed cell alignment along scaffold microfibers while integrating cytocompatible material composites. Precisely, cell alignment refers to the preferred orientation of cells along a defined axis or structural cue, resulting in an organized cellular arrangement rather than a random distribution. Moreover, directional growth refers to the process by which cells grow, migrate, or extend in a specific, guided direction in response to physical, chemical, or mechanical cues in their microenvironment. The use of MNPs in scaffold-guided muscle regeneration offers innovative strategies for promoting effective tissue repair. MNPs allow for the stimulation and alignment of cells within scaffolds when exposed to external magnetic fields, which is crucial for mimicking the natural structural organisation of muscle fibres. External magnetic fields spatially organize MNP-labeled stem cells into anisotropic architectures that recapitulate native skeletal muscle fiber alignment and enhance functional tissue regeneration. It enables the fabrication of highly ordered, biomimetic muscle constructs.[18] In a similar vein, cellular fate is governed by integrin-cytoskeleton-nucleus coupling, along with stiffness, viscoelasticity, poroelasticity, and topography. Atomic force microscopy (AFM),

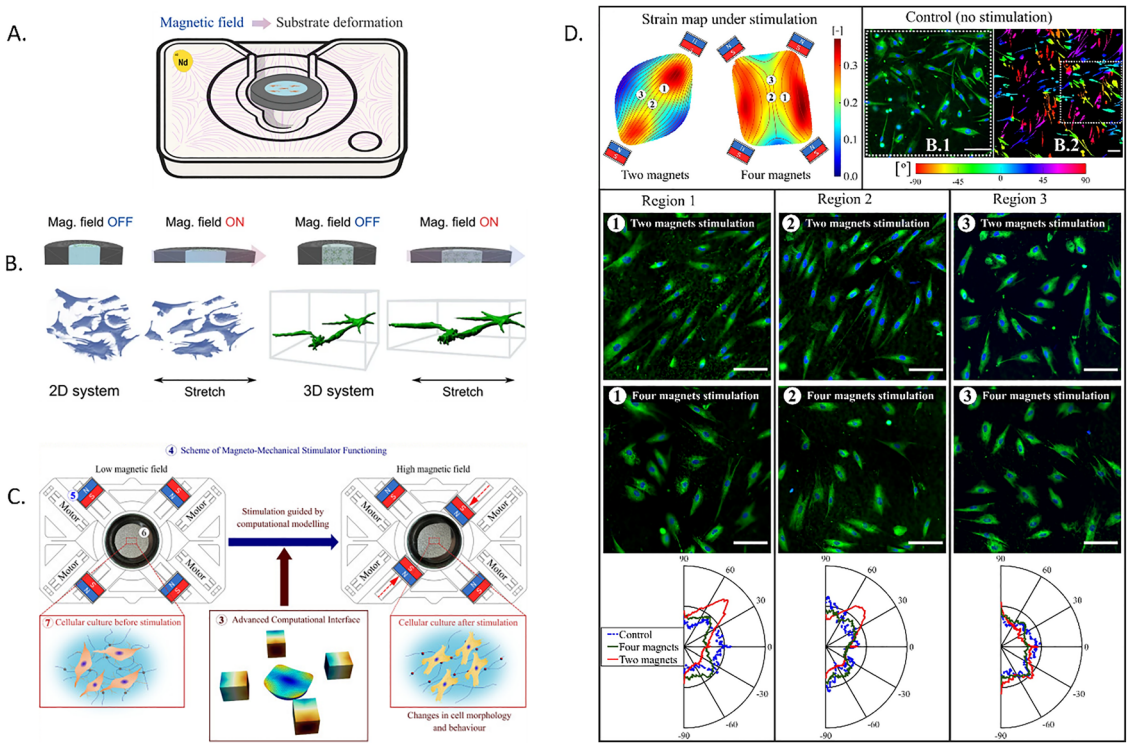

Fig. 2 (A) Schematic of the NeoMag device, featuring integrated arrays of permanent magnets designed to generate precisely controlled magnetic fields. (B) Magneto-mechanical stimulation of astrocytes in 2D and 3D culture systems, showing representative cellular responses induced by dynamic magnetic field switching.[30] (C) Apparatus for magneto-mechanical stimulation connected to the multi-faceted imaging system. (D) Cellular polarization dynamics before and after magneto-mechanical stimulation.[31]

micropipette aspiration, and optical tweezers provide detailed measurements but lack physiological dimensionality and dynamic complexity. Particle-based, substrate-based, and microrobotic systems enable remote, reversible, and spatially programmable force delivery. Thus, magnetic field strength, particle properties, force quantification, and integration with real-time imaging are critical for reproducible mechanobiological studies. Dynamic magnetic actuation recapitulates time-evolving mechanical cues relevant to morphogenesis, fibrosis, and tissue regeneration[30] (Fig. 2A). The unified imaging–stimulation platform offers an adaptable approach for steering advanced *in vitro* investigations into how mechanical forces might influence mechanotransduction-regulated biological responses[31] (Fig. 2B). Moreover, aligned myoblasts can form more effective cell–cell junctions, promoting greater communication and differentiation into mature myotubes. The application of magnetic forces can also influence the orientation of newly formed myotubes, ensuring that they align along the preferred axis of force application, which is essential for functional muscle performance. Scaffolds modified with MNPs not only support alignment but also improve mechanical properties, making them more suitable for load-bearing applications in muscle tissue. Magneto-active constructs established a three-dimensional culture milieu that directed cell alignment along scaffold microfibers while integrating cytocompatible material composites. When combined with matrigel/collagen hydrogels, these constructs further boosted structural organization and maintained cell–matrix interactions inside the engineered microenvironment.[32] Dynamic modulation of intercellular forces during skeletal muscle differentiation programs tissue anisotropy in magnetic matrix actuation (MagMA).[33–35] MagMA-aligned muscle tissues demonstrate coordinated functional maturation and enhanced synchronic contractile twitches within the engineered construct.[36] Similarly, biohybrid microswimmers were fabricated by dip-coating superparamagnetic Fe_3O_4 nanoparticles onto *Chlorella* microalgae, thereby enabling robust magnetic navigation across diverse biological media *via* external magnetic actuation.[37]

In native muscle tissue, muscle fibers are aligned longitudinally to enable efficient force transmission. As such, it is important to recreate this anisotropic environment in tissue engineering. Magnetic nanoparticles (MNPs) can be used to create such alignment when exposed to an external magnetic field. When a magnetic field is applied, MNPs experience magnetic torque and translational forces. When MNPs are dispersed in a hydrogel or polymeric matrix, they tend to align along the magnetic flux and form chain-like structures. As differentiation proceeds, the direction of the newly formed myotubes aligns along the magnetic field axis. This aligned growth helps align contractile proteins such as actin and myosin, thereby increasing the contraction efficiency. If this alignment does not occur, the randomly oriented myotubes would lead to inefficient contraction. Therefore, magnetic guidance not only influences the initial orientation of cells but also influences the macroscopic functional architecture of the regenerated muscle tissue.

Magnetic Fe_3O_4 micro-/nano-stripes were fabricated as alignment inducers within a microchannel-patterned scaffold, enabling responsiveness to *in vitro* stimulation and sustained viability *in vivo* for muscle repair and regeneration.[38] Parameters like stimulation frequency, duration, and field intensity can be tuned across diverse scales and patient-specific conditions, thereby enabling customized therapeutic procedures. Integrating magnetic stimulation with complementary strategies to attain synergistic tissue repair, therefore, represents a promising and clinically applicable approach.[39] Researchers have extensively exploited magnetic field-responsive systems by incorporating diverse magnetic fillers and implementing advanced engineering strategies, achieving notable progress across flexible sensors, magnetic hyperthermia platforms, skeletal muscle regeneration, and bioinspired muscle scaffold development (bionic muscle scaffolds).[40] Static or oscillating magnetic fields modulate gene expression and protein synthesis of myogenic markers (*e.g.*, MyoD, myogenin, and MHC) by inducing mechanobiological and signaling pathway activation.[19]

3.2 Magnetic nanoparticles for drug and growth factor delivery

Briefly, MNPs can serve as effective carriers for bioactive molecules, including growth factors and drugs,[41,42] offering targeted and controlled delivery for various muscle-related diseases.[43–46] Targeted drug delivery to injured muscle tissue *via* magnetic guidance utilises MNPs to carry therapeutic agents directly to the injury site.[46–48] By applying an external magnetic field, MNPs can be precisely directed to concentrate at the affected area, enhancing localised delivery and minimising systemic side effects. This method improves efficacy by enabling higher concentrations and controlled release of therapeutic agents, with real-time monitoring of their delivery *via* magnetic imaging techniques. This approach shows promise for accelerating recovery in muscle injuries and managing chronic muscle disorders.[49] There are numerous muscle diseases, and MNPs could be used for the targeted delivery of drugs or growth factors. Muscle wasting can result from a range of motor neuron disorders that impair nerve signals to muscles, leading to atrophy.[50,51] Additionally, age-related muscle loss (sarcopenia) affects mobility and strength over time, contributing to significant morbidity. Addressing these underlying causes with targeted therapies is essential for preserving muscle function and improving patient outcomes.[52–54] MNPs can be loaded with bioactive molecules (growth factors, mRNA, *etc.*). They can be activated to release their payload when exposed to an external magnetic field, enabling precise modulation of the release rate. This approach ensures localised action, minimising systemic exposure and maximising therapeutic efficacy, as higher drug concentrations are delivered directly to the site of healing.[55] By promoting sustained release, these systems stimulate myoblast proliferation and differentiation, leading to improved muscle regeneration and recovery outcomes in various muscle injuries and degenerative conditions.[56–59] MNP-based delivery systems offer precise, controlled release of bioactive molecules, improving therapeutic efficacy while minimising systemic side effects. These advanced delivery methods hold promise for treating muscle-related conditions by promoting tissue regeneration and reducing muscle degeneration. Delivery methods for MNPs include targeted injection at the injury site or implantation within scaffolds, enabling precise, localised delivery and sustained release of therapeutic agents to enhance muscle repair and regeneration.[60–62]

3.3 MNPs in gene therapy

Magnetic nanoparticles (MNPs) can deliver plasmid DNA or RNA (*e.g.*, siRNA and mRNA) encoding therapeutic proteins, such as myogenic factors, to promote muscle regeneration and prevent atrophy.[63] Using magnetofection, MNPs enhance cellular uptake and transfection efficiency *via* magnetic fields, thereby improving targeted delivery of genetic materials. It can also be used to co-deliver bioactive agents such as growth factors and gene therapy vectors to enhance muscle regeneration synergistically.[64–66] Using magnetic targeting, MNPs ensure the localised release of these combined therapies at the site of muscle injury or degeneration, improving treatment precision and efficacy.[67] By facilitating stem cell and gene therapies, MNPs enhance the potential for effective muscle regeneration and recovery.[68–70]

4. Magnetic nanoparticles in skeletal muscle tissue engineering: current applications and research

4.1 Magnetic nanoparticle-based scaffolds and cell culture models

In vitro studies are essential for evaluating the potential of MNPs for muscle regeneration, as they provide controlled environments to assess interactions between MNPs and muscle cells.[71,72] MNPs can be integrated into a range of biomaterial scaffolds such as hydrogels, electrospun nanofibers, and 3D-bioprinted matrices, to enhance myoblast proliferation, promote differentiation, and guide cellular alignment, all of which are critical for the formation of functional muscle tissue. Electrospinning has been widely used to fabricate nanofibrous scaffolds resembling the natural extracellular matrix (ECM) of muscle tissue. When MNPs are embedded within polymeric nanofibers (*e.g.*, polycaprolactone (PCL) or gelatin), the scaffolds exhibit enhanced mechanical strength, electrical conductivity, and bioactivity, promoting muscle satellite cell differentiation.[73,74] Hydrogels such as alginate, gelatin, or collagen provide a hydrated environment suitable for muscle cell growth. MNP-infused hydrogels respond to external magnetic fields, allowing for the controlled orientation and migration of myoblasts, which is crucial for forming aligned muscle fibres[75] (Fig. 3A). Advances in 3D bioprinting have enabled the fabrication of complex MNP-loaded bio-inks, allowing the creation of muscle-like tissue constructs with tunable mechanical, electrical, and biochemical properties. These models mimic native muscle architecture, thereby improving myogenic differentiation.[76]

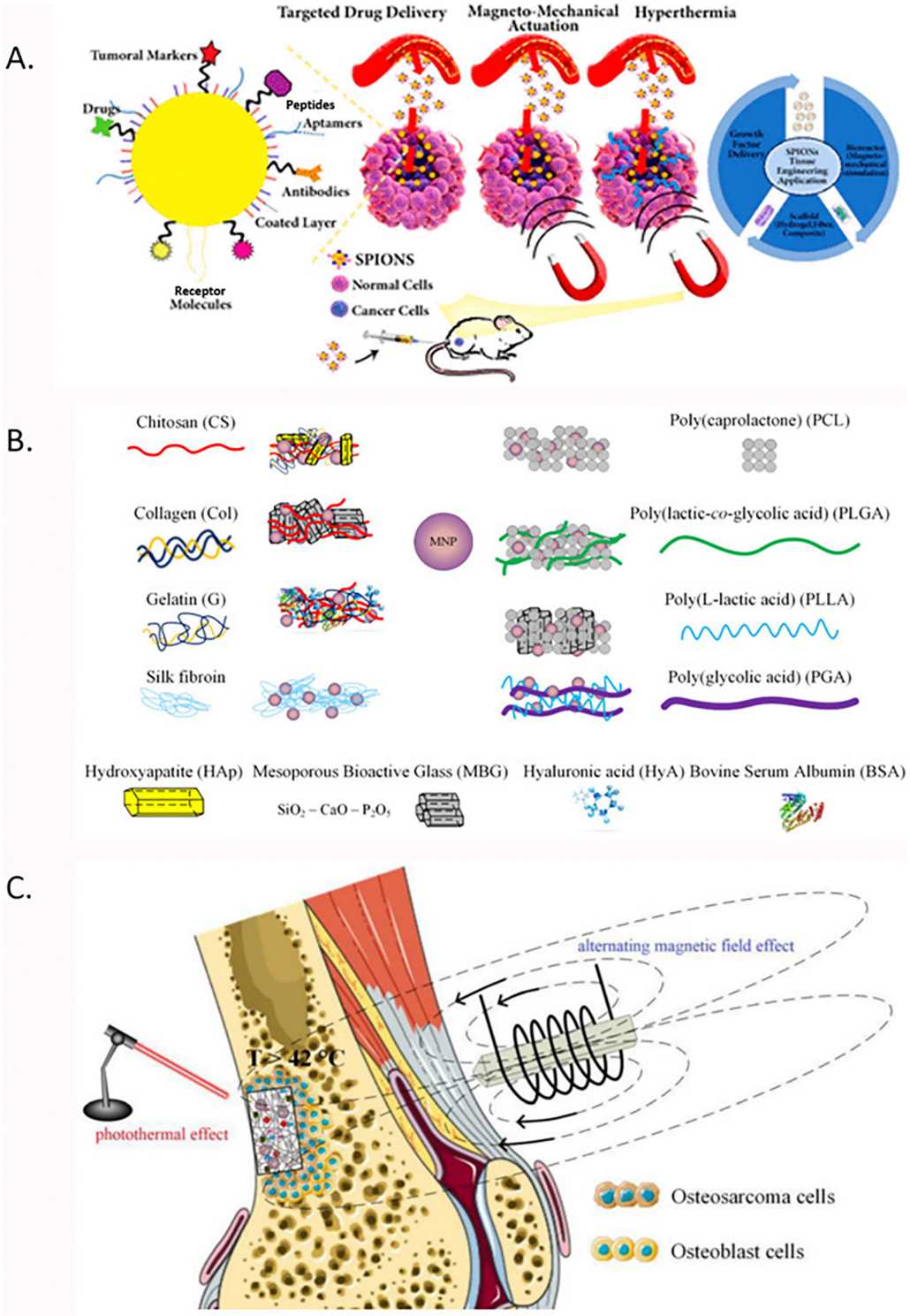

Fig. 3 (A) Superparamagnetic iron oxide nanoparticles (SPIONs) in tissue engineering and other related applications.[75] (B) Different hydrogel and polymer compositions for skeletal tissue engineering. (C) Magnetic hyperthermia and photothermal therapy treatment for oncological disease in BTE.[77]

The integration of MNPs into muscle tissue engineering has demonstrated several significant advantages. First, MNP-based scaffolds provide topographical and biochemical cues that promote myoblast fusion and myotube maturation. Furthermore, the use of magnetic fields enables non-invasive control over cell alignment, migration, and differentiation, thereby improving tissue organisation. MNPs also help to reinforce biomaterial scaffolds, enhancing their mechanical strength, conductivity, and stability. However, for MNPs to be widely used, some limitations must be addressed. High concentrations of MNPs can generate reactive

oxygen species (ROS), causing oxidative stress and cellular damage. Although the mechanism for this is largely unknown, factors to mitigate these issues need to be explored. The magnetic properties of MNPs may diminish over time, affecting their effectiveness in prolonged tissue regeneration. This will have implications for 'off-the-shelf' products and for quality control of materials that will need monitoring. The incorporation of MNPs into large scaffolds for critical-size defects requires precise control over nanoparticle distribution, posing manufacturing challenges. Indeed, it is possible to generate large volumes of MNPs; however, successfully integrating and distributing them within large scaffolds is somewhat challenging.[21]

4.2. MNPs in combination with biomaterial scaffolds and testing in animal models

Integrating MNPs into various biomaterial scaffolds to improve their efficacy in muscle repair has also been demonstrated successfully.[78,79] For example, injectable magnetic hydrogels deliver stem cells and growth factors to injured muscle sites with precise control under external magnetic fields. Electrospun magnetic fibres have also been used to provide a structural framework mimicking muscle ECM, guiding cell orientation and differentiation[77] (Fig. 3B and C). The improvement in the mechanical properties of muscle tissue scaffolds has been demonstrated using biodegradable polymer composites, which ensure gradual degradation and integration into the host tissue.[80] Preclinical studies using animal models have been instrumental in demonstrating the effectiveness of MNP-based approaches in muscle tissue repair. Rodent models are commonly used due to their similarities to human muscle physiology and well-established injury models. These studies have shown some promising results. MNP-containing scaffolds implanted in muscle defects have shown accelerated muscle fibre regeneration, underpinned by increased myogenic gene expression. MNPs, when combined with angiogenic factors (*e.g.*, VEGF), have also been shown to promote blood vessel formation, a critical aspect of muscle regeneration and repair. MNPs have also been used as nanocarriers for myogenic growth factors (*e.g.*, IGF-1 and FGF-2), enabling localised delivery and sustained release, further improving muscle regeneration (Table 2).[81]

Table 2 Magnetic nanoparticles in skeletal muscle tissue engineering applications and research

S. no.	Application	Type of MNP or system	Mechanism/role	Outcome/significance	Reference (year)
1	Magnetic-assisted alignment of myoblasts	Magnetite MNPs	Magnetically directed cell orientation	Organised myotube formation	McCarthy *et al.* (2007)[16]
2	Cell density enhancement	Internalised magnetite nanoparticles	Improved packing *via* magnetic force	Slight reduction in fibre thickness	Kirihara *et al.* (2021)[76]
3	Magnetic nanofibrous scaffolds	Electrospun magnetic scaffolds	External field combined with scaffolds	Accelerated muscle regeneration	Hu *et al.* (2022)[72]
4	Artificial muscle *via* magnetic force tissue engineering	Magnetic force TE	Magnetically manipulated tissue formation	Functional muscle constructs	Nabavinia *et al.* (2020)[75]
5	Biomaterials functionalized with MNPs	MNP-integrated hydrogels/ scaffolds	Cell guidance and control	Enhanced precision in tissue engineering	Goranov *et al.* (2024)[80]
6	Magnetic-based tissue engineering (MagTE)	Various magnetic particles	Non-invasive mechanical/ thermal stimulation	Fine-tuned tissue control	Xia *et al.* (2018)[82]
7	Magnetic scaffolds for mechanotransduction	Magnetic scaffolds	Actuation *via* magnetic fields	Precise control of tissue formation	Paltanea *et al.* (2023)[77]
8	Magnetic micro swimmers	Biohybrid micro swimmers coated with MNPs	Magnetic guidance	Targeted delivery in the muscle context	Liu *et al.* (2022)[38]
9	Magnetic 3D bioprinting	Iron oxide/Au MNPs + magnetism	Rapid cell printing into structures	Efficient, biomimetic constructs	Yang *et al.* (2024)[83]
10	3D cell culturing by magnetic levitation	Iron oxide/Au MNPs + peptides	Levitation *via* a field	Scaffold-free 3D tissue models	Jain *et al.*, (2024)[6]
11	Magnetoreception for gene delivery	MNP-nucleic acid complexes	Magnetic cell transfection	Enhanced gene uptake in muscle cells	Li *et al.*(2025)[67]
12	Magnet-assisted transfection	Iron oxide nanoparticles	Magnetic nucleic acid delivery	Efficient transfection	Gupta *et al.* (2007)[14]
13	MSC tracking and delivery	MNP-labelled MSCs	Magnetic navigation and imaging	Improved homing and integration	Coletti *et al.* (2007)[58]
14	Nanomedicine overview	MNPs in nanomedicine	Imaging, sensing, therapy	Broad biomedical roles	Colapicchioni *et al.* (2022)[19]
15	Mechanisms under magnetic fields	MNP interactions	Mechanical/thermal effects on cells	Insight into cellular mechanisms	Elahi *et al.* (2021)[84]
16	Magnetic hydrogels for triggered release	Iron-oxide embedded hydrogels	AC field-induced heating	Controlled drug release	Pourmadadi *et al.* (2022)[48]
17	Skeletal muscle regeneration nanomaterials	Various nanomaterials	Supporting regeneration	Overview of nanoscale strategies	Jeong *et al.* (2022)[85]
18	Nanomedicine tool for muscle disorders	Nanomaterials in muscle pathology	TE and disease targeting	Broad application coverage	Colapicchioni *et al.* (2022)[19]
19	Nanobiofabrication techniques	Nanomaterials + fabrication	Material, cell integration	Enhanced regeneration platforms	Han *et al.* (2023)[30]
20	Electro-spun anisotropic magnetic scaffolds	SF/MNP blends	Fiber alignment	Mimicking natural muscle anisotropy	Yang *et al.* (2024)[83]

4.3. Biocompatibility and long-term effects of MNPs *in vivo*

One of the most critical challenges in using MNPs for SMTE is ensuring their biocompatibility and minimising long-term adverse effects. Several factors including particle size, composition, surface functionalisation, and aggregation tendencies influence the biological response to MNPs. Studies have demonstrated that uncoated or improperly coated MNPs can trigger oxidative stress, inflammatory responses, and even apoptosis in muscle cells. Additionally, the interaction of MNPs with cellular organelles such as mitochondria can disrupt metabolic processes, potentially leading to long-term dysfunction.

Functionalising MNPs with biocompatible coatings, such as polyethylene glycol (PEG), dextran, or silica, can enhance their stability and reduce toxicity. Moreover, hybrid nanoparticles incorporating biodegradable polymers or biomimetic coatings have been investigated to improve MNP biocompatibility. However, long-term *in vivo* studies are needed to assess the chronic effects of MNP exposure on muscle tissue and surrounding organs.

4.4. Potential accumulation and clearance issues

MNP clearance from the body is another major concern. Once administered, MNPs can accumulate in organs such as the liver, spleen, and kidneys, leading to potential toxicity over time. The reticuloendothelial system (RES) plays a key role in clearing MNPs from circulation, but the clearance efficiency varies with particle size and surface properties. Large or aggregated MNPs tend to be retained in tissues longer, increasing the risk of long-term adverse effects. Researchers are exploring the development of MNPs with biodegradable cores, such as iron oxide nanoparticles coated with bioresorbable polymers, to facilitate clearance. Enzymatic degradation and dissolution into non-toxic byproducts are being investigated as potential solutions. Nevertheless, designing MNPs that completely degrade within a defined timeframe without compromising their functional properties remains a significant challenge.

5. Optimisation of magnetic field parameters for effective cell growth

In skeletal muscle tissue engineering, the use of external magnetic fields is essential for controlling the behaviour of MNPs. However, optimising magnetic field parameters such as strength, frequency, and gradient remains a significant challenge. The effectiveness of MNP-mediated stimulation depends on precise control over these parameters to induce desired cellular responses such as enhanced proliferation, differentiation, and alignment of muscle cells. The effectiveness of magnetic stimulation in tissue engineering depends on the accurate optimization of magnetic field parameters, including intensity, frequency, waveform, exposure time, and spatial gradient. The parameters must be selected depending on the type of tissue being engineered, the physicochemical properties of the magnetic nanoparticles (MNPs), and the biological tolerance of the target cells. In most cases, a low to moderate magnetic field intensity (1–100 mT for static fields and a few kA m^{-1} for alternating fields) is sufficient for tissue engineering applications to align cells, stimulate proliferation, and activate mechanotransduction signaling pathways without causing excessive heating or oxidative stress. The magnetic field must exert sufficient magnetic torque or translational force on the MNPs while remaining below cytotoxic and thermal safety thresholds. The choice of frequency is a critical determinant of the type of interactions between nanoparticles and cells. Low-frequency alternating magnetic fields ($\leq$1 kHz) are commonly used for dynamic mechanostimulation, cytoskeletal remodeling, and differentiation promotion, especially in bone and cartilage tissue engineering. Higher frequencies (100–500 kHz) are mainly used for applications requiring mild hyperthermia or antimicrobial properties, as they facilitate Néel and Brownian relaxation, resulting in controlled hyperthermia. To prevent irreparable tissue damage, temperature increases in these applications should be limited to the mild hyperthermia range (39–43 °C). Static magnetic fields are used for long-term cell orientation and differentiation. In contrast, pulsed electromagnetic fields can modulate ion channel activities, intracellular calcium signaling, and gene expression involved in osteogenic and chondrogenic differentiation. Parameter selection should be systematic and application-specific, combining material properties (saturation magnetization, particle size, and surface functionalization), biological response variables (cell viability, differentiation, and cytokine expression), and safety thresholds consistent with international exposure limits. A parameter-response matrix relating field strength and frequency to biological endpoints can improve reproducibility and facilitate comparisons across studies. The addition of normalized parameter, including the magnetic-to-thermal energy ratio ($\mu_0 mH/kT$) significantly strengthens the rationale for the design of magnetic stimulation protocols. By clearly articulating these criteria, magnetic field-assisted methods can be improved for translation from laboratory-scale experiments to tissue engineering applications.[84,86,87]

5.1 Limitations in achieving uniform magnetic fields in complex biological systems

One of the key limitations of using MNPs for SMTE is the difficulty in achieving a uniform magnetic field distribution within complex biological environments. Skeletal muscle tissue comprises multiple cell types, extracellular matrix components, and vascular networks, all of which can influence magnetic field interactions.[88,89] Non-uniform fields can result in localised stimulation, leading to inconsistent muscle regeneration. To address this issue, researchers are developing advanced magnetic field delivery systems including dynamic or rotating magnetic fields and multi-axial field generators. Computational modelling techniques are also being employed to simulate field interactions and optimise delivery strategies. Despite these advancements, achieving consistent and reproducible magnetic field exposure *in vivo* remains a significant hurdle.[90]

5.2 Challenges in the large-scale synthesis of MNPs

For MNP-based approaches to be clinically viable, scalable, and cost-effective, synthesis methods must be established.

Currently, the most common synthesis techniques include co-precipitation, thermal decomposition, and microemulsion methods.[91–93] Each method presents specific challenges in achieving uniform particle size, shape, and magnetic properties. Batch-to-batch variability is a major issue in large-scale synthesis, as small deviations in reaction conditions can lead to inconsistencies in MNP properties.[94] To address this, continuous flow synthesis techniques and automated manufacturing processes are being explored. These approaches offer better control over reaction parameters and improve reproducibility. However, the transition from laboratory-scale production to industrial-scale manufacturing remains a significant challenge.[19,95]

5.3 Issues with reproducibility and standardisation for clinical applications

The lack of standardised protocols for MNP production, characterisation, and functionalisation poses a significant barrier to clinical translation. Variability in surface coatings, magnetic properties, and biocompatibility between batches can affect therapeutic efficacy and safety. Regulatory agencies require stringent quality control measures to ensure consistency in clinical-grade MNPs. Collaborative initiatives between academia, industry, and regulatory bodies are crucial for defining best practices in MNP manufacturing and clinical application.[96,97] Despite these challenges, ongoing research is focused on overcoming current limitations to enable the safe and effective use of MNPs in SMTE. Some potential solutions include developing fully biodegradable MNPs by coating to enhance clearance and minimise long-term toxicity, and utilising computational modelling and real-time feedback systems to optimise magnetic field exposure for muscle regeneration. Scalable manufacturing techniques are used for implementing continuous flow synthesis and automated quality control to improve reproducibility and standardisation and to establish clear guidelines for the clinical translation of MNP-based therapies to ensure safety and efficacy. Addressing these issues requires interdisciplinary collaboration between materials scientists, bioengineers, and clinicians.[85,98,99] Future research should focus on developing safer, more effective MNP-based strategies to advance muscle tissue engineering and bring these technologies closer to clinical applications.[100,101]

5.4 Challenges in translating MNP-based muscle regeneration to applications in humans

Magnetic nanoparticles (MNPs) have potential for muscle regeneration therapies. However, several obstacles prevent their translation from animal models into applications in humans.[102,103] Biological challenges include species-specific variations in cell responses to MNPs, which complicate the predictive power of animal studies.[104–107] In addition, the long-term biocompatibility and potential toxicity of MNPs in human tissues remain inadequately evaluated, raising concerns about their safety in clinical environments.[108,109] Ethical considerations related to the use of animal models to test MNPs also complicate the survey scenario, as regulations governing the humane treatment of animals are still being tightened. Translational research should navigate these ethical dilemmas, ensuring that the findings are ethically derived.[82,110] Finally, technological challenges arise from the complexity of the MNP manufacturing process and the need for standardised production methods that guarantee reproducibility and efficacy in different biological systems.[111] Together, these challenges highlight the multifaceted barriers that must be addressed for successful translation in human therapies.[33]

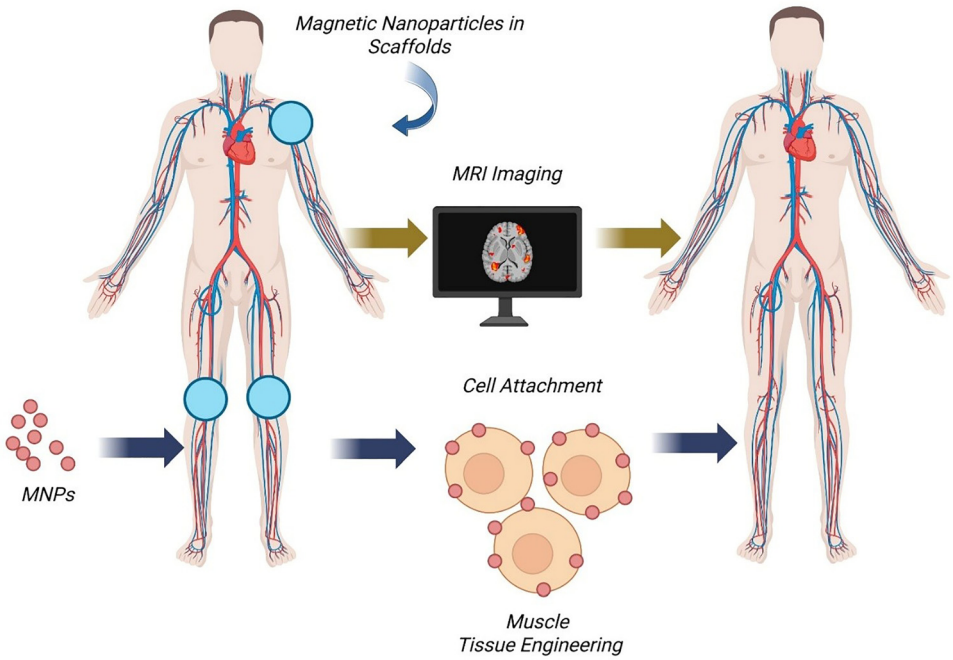

Fig. 4 Schematic of the magnetic nanoparticles (MNPs) incorporated into scaffolds for muscle tissue engineering, enabling MRI imaging, enhanced cell attachment, and targeted tissue regeneration.

5.5. Next-generation magnetic nanoparticles and advanced magnetic field applications

A significant advancement in MNP technology involves the development of multifunctional nanoplanks.[34] These next-generation MNPs are engineered to perform multiple roles including imaging, targeted drug delivery,[35] and tissue technology support.[112,113] By integrating these functionalities into a single platform, researchers aim to develop more efficient and targeted medical solutions. Another promising direction involves the development of biomimetic constructs that integrate MNPs with advanced polymers or hydrogels. These materials increase the mechanical and electrical properties of scaffolds used in regenerative therapy, especially in applications such as nerve and muscle tissue engineering. A combination of MNP with biocompatible hydrogel also improves cell attachment and spreading, thereby enhancing their overall therapeutic efficacy.[114]

Magnetic field technologies are also advancing continuously to enable more controlled, accurate applications in weaving technology. Wearable and implantable devices are developed to enhance the targeted activation of MNPs in the body by generating localised magnetic fields. These systems have the potential to facilitate real-time medical intervention without aggressive procedures.[83,115] Another key innovation is the development of real-time surveillance and control systems that utilise magnetic field areas to regulate tissue regeneration. By dynamically tailoring the magnetic environment, these systems can support cell proliferation, differentiation, and tissue repair, paving the way towards more efficient and personalised medical approaches.[116]

Integrating MNPs with emerging biomedical technologies is expected to unlock new opportunities in regenerative medicine (Fig. 4). A promising application lies in the use of 3D bioprinting for muscle tissue scaffolding. MNPs can be incorporated into bio-inks to respond to external magnetic fields, enabling the fabrication of structured scaffolds and guiding cellular organisation and tissue formation with high spatial precision.[117] The future of MNPs in tissue engineering lies in their capacity to integrate with diverse materials, advanced magnetic field applications, and next-generation biomedical technologies. Enhancing their functionality, scalability, and accuracy is the key to developing more effective regenerative therapies. As innovation in this field continues to grow, MNPs are poised to play a pivotal role in shaping the next generation of biomedical applications, ultimately contributing to improved patient outcomes and the emergence of novel treatment strategies.

6. Conclusion and future directions

MNPs can revolutionize tissue technology, drug delivery, and regenerative medicine. However, successful clinical translation depends on overcoming many important challenges. Suitable studies should be conducted to assess safety, including toxicity, biophysical properties, and long-term accumulation, to ensure that these nanoparticles do not pose a risk to human health. Progress in surface modification and biodegradable coating technologies can help reduce these concerns and increase biological diversity. In addition, accurate control of magnetic fields is an important technical barrier. Effective magnetic field design is necessary to guide MNP to target sites and to ensure consistent behaviour across complex biological systems. Developing more sophisticated field-control mechanisms such as advanced electromagnetic systems and appropriate nanodoping can enhance the efficacy of MNP-based treatments. Scalability and standardisation are equally important in the production of nanomaterials for clinical applications. The ability to produce MNPs with consistent quality and functional properties on an industrial scale is important for realizing their wide range of applications. The implementation of regulatory guidelines and standardised synthesis methods will ensure product quality and facilitate approval for medical use. As research on magnetic nanoparticles (MNPs) advances, numerous emerging directions are expanding in tissue technology and regenerative therapies. This development aims to enhance functionality, accuracy, and integration with state-of-the-art technologies to achieve improved clinical outcomes. Advancing biomaterials for skeletal muscle regeneration requires sustained research and development efforts to address current obstacles and enhance clinical applicability. Short-term, immediate goals should focus on establishing robust proof-of-concept arrangements for safe, efficient, targeted delivery, preliminary immune modulation, and controlled cell guidance in well-defined *in vitro* and small-animal models. Priority should be given to optimizing nanoparticle physicochemical properties, improving magnetic field precision and reproducibility, and integrating real-time imaging for basic theragnostic validation. Standardizing synthesis protocols, biocompatibility testing, and functional performance metrics will be essential to ensure reproducibility and accelerate translational readiness. In the long term, the field should advance toward fully integrated, intelligent MNP-based platforms that combine targeted delivery, bionics, immune modulation, cell guidance, and real-time diagnostics within a single, scalable system. Such multifunctional constructs could enable organ preservation strategies, promote *in situ* tissue regeneration, and dynamically adapt therapeutic outputs in response to physiological feedback. Coupling MNP technologies with artificial intelligence–driven control systems would further allow predictive modeling, personalized field modulation, and closed-loop therapeutic regulation. The convergence of nanotechnology, regenerative medicine, and AI-enabled precision control, therefore, represents a transformative direction for next-generation magnetically actuated biomedical platforms. Furthermore, interdisciplinary collaboration between physicists, biomedical engineers, and doctors will be necessary to solve these challenges. Constant research, technological innovation, and regulatory progress will pave the way for tissue technology and beyond MDP's safe and more effective applications. By overcoming these boundaries, MNP-based treatment can bring infection control solutions from the real world and experimental research, eventually improving patient outcomes across different fields of health services.

Conflicts of interest

There are no conflicts to declare.

Abbreviation

SMTE	Skeletal muscle tissue engineering
MNPs	Magnetic manoparticles
ECM	Extracellular matrix
VML	Volumetric muscle loss
iPSCs	Induced pluripotent stem cells
MSCs	Mesenchymal stcm Cells
TESkM	Tissue engineering of skeletal muscle
MDCs	Muscle-derived cells
ESCs	Embryonic stem cells
MRFs	Myogenic regulatory factors
BMPs	Bone morphogenetic proteins
PCL	Polycaprolactone
PEG	Polyethylene glycol
RES	Reticuloendothelial system

Data availability

No primary research results, software or code has been included and no new data were generated or analysed as part of this review.

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