Review article

Fostering tissue engineering and regenerative medicine to treat musculoskeletal disorders in bone and muscle

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ABSTRACT

The musculoskeletal system, which is vital for movement, support, and protection, can be impaired by disorders such as osteoporosis, osteoarthritis, and muscular dystrophy. This review focuses on the advances in tissue engineering and regenerative medicine, specifically aimed at alleviating these disorders. It explores the roles of cell therapy, particularly Mesenchymal Stem Cells (MSCs) and Adipose-Derived Stem Cells (ADSCs), biomaterials, and biomolecules/external stimulations in fostering bone and muscle regeneration. The current research underscores the potential of MSCs and ADSCs despite the persistent challenges of cell scarcity, inconsistent outcomes, and safety concerns. Moreover, integrating exogenous materials such as scaffolds and external stimuli like electrical stimulation and growth factors shows promise in enhancing musculoskeletal regeneration. This review emphasizes the need for comprehensive studies and adopting innovative techniques together to refine and advance these multi-therapeutic strategies, ultimately benefiting patients with musculoskeletal disorders.

1. Introduction

The musculoskeletal system comprising the bones, muscles, joints, cartilage, ligaments, tendons, and other connective tissues, plays a crucial role in human mobility and structural support [1]. Musculoskeletal disorders include various conditions resulting from underlying immunological, genetic, and environmental factors [2]. Musculoskeletal disorders, including rheumatoid arthritis and spondyloarthropathies, are caused by immunological factors and typically result from immune system dysfunction, wherein the body's immune response mistakenly targets its own tissues [3]. Muscular dystrophy and osteogenesis imperfecta exemplify how genetic mutations can compromise muscle and bone strength [4]. Conditional disorders arise mainly from external factors, aging, lifestyle choices, or other non-genetic and non-immunological influences (Fig. 1 and Table 1) [5]. Osteoarthritis is the most prevalent conditional degenerative joint disease affecting the articular cartilage in joints. It culminates in pain, stiffness, and restricted joint mobility, impacting more than 500 million adults worldwide [6].

Similarly, osteoporosis, characterized by reduced bone density, renders the bones brittle and susceptible to fractures [7]. Overuse or repetitive strain can lead to conditions like tendinitis and bursitis, causing pain and inflammation in the affected tendons or bursae in muscles [8]. Disorders such as osteoarthritis and muscle-wasting conditions like muscular dystrophy substantially impact patients' lives and impose substantial economic burdens on healthcare systems [9–11]. These conditions affect children and adults, considerably reducing life expectancy and the quality of life [12,13].

Despite the critical role of the musculoskeletal system, its capacity for self-repair is limited, presenting challenges in treating musculoskeletal disorders [14]. For example, articular cartilage has limited self-repair capacity due to its lack of blood vessels, which hinders the supply of reparative cells and nutrients [15]. Similarly, bones possess some regenerative ability, but it is often insufficient to fully recover from significant defects or severe osteoporosis [16]. In genetic conditions like muscular dystrophy, muscles progressively deteriorate and lack robust regenerative mechanisms to counteract the disease's advance [17,18]. Therefore, innovative therapeutic interventions are required to bridge

Abbreviations

ADSCs	Adipose-Derived Stem Cells
AKT	Protein Kinase B
ALP	Alkaline Phosphatase
BMPs	Bone Morphogenetic Proteins
BP	Bone Powder
CaP	Calcium Phosphate
CCR2	*C*–C Motif Chemokine Receptor 2
CS	Chitosan
ECM	Extracellular Matrix
ERK	Extracellular Signal-Regulated Kinases
ESCs	Embryonic Stem Cells
FGF	Fibroblast Growth Factor
GelMA	Gelatin Methacryloyl
HAp:	Hydroxyapatite
HGF	Hepatocyte Growth Factor
IDO	Indoleamine 2,3-Dioxygenase
IFNγ	Interferon Gamma
IGF	Insulin-like Growth Factor
IL:	Interleukin
iPSCs	Induced Pluripotent Stem Cells
MAPK	Mitogen-Activated Protein Kinases
MHC	Major Histocompatibility Complex
MMPs	Matrix Metalloproteinases
MSCs	Mesenchymal Stem Cells
NKs	Natural Killer Cells
NMDA	*N*-Methyl-D-Aspartate
OCN	Osteocalcin
OGP	Osteogenic Growth Peptide
OPN	Osteopontin
PCL:	Polycaprolactone
PDGF	Platelet-Derived Growth Factor
PI3K	Phosphoinositide 3-Kinases
PRP	Platelet-Rich Plasma
RUNX2	Runt-related Transcription Factor 2
SIS	Small Intestinal Submucosa
TGF-β:	Transforming Growth Factor-beta
TNF-α:	Tumor Necrosis Factor Alpha
VEGF	Vascular Endothelial Growth Factor

the gap between the body's limited healing capacity and the clinical need for full functional recovery [18].

Tissue engineering and regenerative medicine are vital fields in this context, offering strategies that can enhance or replace the natural healing processes [19]. By integrating cell therapy (such as stem cells), bioactive materials (hydrogels and scaffolds), growth factors (such as transforming growth factor-beta [TGF-β] and vascular endothelial growth factor [VEGF]), and external stimulations (electricity and ultrasound), these interdisciplinary approaches aim to restore the lost function and structure of damaged tissues [20]. Stem cells, including mesenchymal stem cells (MSCs) [21] and adipose stem cells (ADSCs) [22], are central to cell therapy in regenerative medicine. Stem cells can differentiate into various cell types, including bone and muscle cells. Biomaterials are crucial for tissue engineering and provide structural
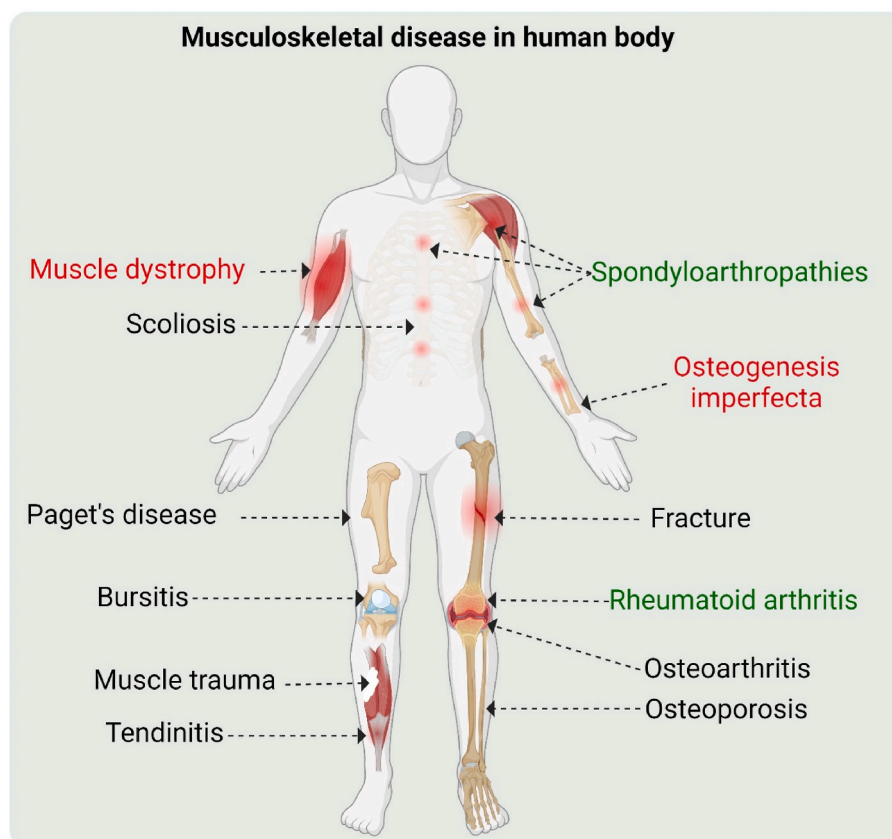
Fig. 1. Specific areas of the human body affected by different musculoskeletal disorders. Red, green, and black text indicate musculoskeletal diseases caused by the immune system; genetic factors; and social, behavioral, and environmental condition, respectively.

Table 1
Etiologic classification of different musculoskeletal diseases.

Etiologic classification	Name of disorder	Description of the disorder
Immunological disorders	Rheumatoid arthritis	Autoimmune disease causing chronic joint inflammation, pain, and damage.
	Spondyloarthropathies	Conditions like ankylosing spondylitis and psoriatic arthritis are characterized by immune-mediated inflammation of the spine and sometimes peripheral joints.
Genetic disorders	Muscular dystrophy	Group of disorders that cause progressive muscle weakness and degeneration due to mutations in muscle proteins.
	Osteogenesis imperfecta	Characterized by brittle bones that fracture easily.
Conditional disorders	Osteoarthritis	Develops from wear and tear on joints, leading to cartilage degradation and joint dysfunction.
	Osteoporosis	Characterized by reduced bone mass and density, increasing fracture risk. It is often associated with aging or hormonal changes.
	Tendinitis and bursitis	Conditions resulting from overuse or repetitive strain causing inflammation and pain in tendons or bursae.
	Scoliosis	An abnormal curvature of the spine arising from congenital, neuromuscular, or idiopathic factors.
	Paget's disease	Disrupts normal bone remodeling, leading to enlarged and misshapen bones.
	Fractures and traumatic injuries	Caused due to acute mechanical forces; these injuries are not typically related to genetic or immune factors.

support to facilitate tissue growth and regeneration [23]. Owing to advancements in material science, biocompatible materials have been developed that closely mimic the properties of native tissues, providing an ideal environment for cells to thrive and differentiate [24]. Biomolecules such as signaling molecules, growth factors, and external stimulations are instrumental in regenerative medicine [25,26]. These tools can guide cell behavior, promote tissue growth, and modulate immune responses. The efficacy of regenerative treatments can be enhanced by precisely controlling the release and interactions of biomolecules [27]. Therefore, these techniques can be employed to address musculoskeletal disorders; the treatment approach can be chosen based on the specific disorder, its stage, and the patient's condition.

Effective collaboration between scientists, clinicians, and researchers from diverse fields, such as biology, materials science, engineering, and clinical medicine, is essential for developing and implementing clinically applicable tissue engineering and regenerative medicine techniques [28,29]. However, despite efforts to advance research, the findings have not yet been practically applicable [30]. While acknowledging the significant gap between academic research and clinical applications in musculoskeletal treatment [31], this review aims to comprehensively delineate the current landscape of tissue engineering and regenerative medicine. We recognize that bridging this gap is a gradual process, requiring the cumulative efforts of the entire scientific community; therefore, we have compiled the latest advancements and innovations in the field. This can support the ongoing translational efforts and provide a foundational resource for researchers from diverse disciplines who are new to this study area. We observed a lack of concise and accessible reviews offering foundational knowledge. Consequently, the present review was crafted as a "ready to go" resource offering clarity and insight, facilitating a smoother transition for researchers venturing into this complex yet fascinating domain.

2. Bone and muscle regeneration in the human body

Injuries to bones and muscles are among the most common tissue injuries [32]. Both tissues can regenerate themselves quite effectively. Once complete, bone healing often restores the bone to its uninjured state [33]. However, severe damage can only be remodeled over a prolonged period. Similarly, even though muscle tissues can recover quickly, tissue engineering approaches are necessary under severe loss of muscle volume [34]. Therefore, bone and muscle recovery must be thoroughly understood to develop an effective regenerative treatment scheme.

2.1. Bone regeneration

The bone provides structure to the body, protects internal organs, and enables movement along with muscles [35]. The bone substrate comprises proteins, calcium, and minerals, including collagen. Bones can self-heal and regenerate via a complex process involving several

mechanisms and cellular interactions (Fig. 2). Briefly, stem cell-derived osteoclasts reabsorb damaged bones during bone remodeling to create space; the osteoblasts fill this space and reconstruct the bone tissue, restoring bone structure and strength [36]. However, bone regeneration can span weeks to months, depending on the degree of injury and the individual's ability to heal. The initial phase of bone healing occurs in the first week after injury, during which a hematoma forms at the fracture site [37]. This stage triggers the coagulation cascade and a series of pro- and anti-inflammatory events [38]. Key cytokines such as interleukin (IL)-1, IL-6, TNF-α, and growth factors like VEGF and RANKL orchestrate the inflammatory response, attracting M1 and M2 macrophages, Th1 and Th2 cells, and fibroblasts to the injury site [36].

During the second and third weeks, soft callus formation is observed, during which angiogenesis is critical. This process is driven by IL-6, TNF-α, VEGF, and RANKL and involves the active participation of endothelial cells, hypertrophic chondrocytes, and osteoblasts. These cells form a soft matrix that stabilizes the fracture [38].

The transition from soft to hard callus occurs between weeks 4 and 17. During this period, the callus undergoes matrix mineralization and woven bone is observed [39]. This phase is marked by the activity of cytokines and growth factors such as IL-6, TGF-β, VEGF, and matrix metalloproteinases (MMPs). The cellular players in this phase include endothelial cells, osteocytes, osteoblasts, and osteoclasts, all contributing to the gradual hardening of the bone matrix.

The final phase of bone healing from weeks 18–52 involves remodeling. This phase is crucial to further heal the fracture and restore the functional structure of the bone. TGF-β and MMPs play significant roles, influencing the activities of endothelial cells, osteocytes, osteoblasts, and osteoclasts.

Thus, fractured bones exhibit self-healing ability and return to their original state via cellular regulation; however, approximately 10 % of fractures do not heal [40], and delayed healing causes secondary problems [35,41]. Therefore, techniques involving bone grafts, biomaterials, and growth factors are required to assist bone healing.

2.2. Muscle regeneration

Muscles are tissues attached to bones. They contract during exercise or to maintain posture. They are attached to blood vessels and internal organs, providing protection and enabling movement. Unlike in other tissues, the stem cell population in the tissue replaces damaged muscles, preventing further muscle loss and promoting regeneration [43]. Mild injuries can spontaneously heal, whereas severe injuries incur tissue loss and deformation [44]. Muscle damage caused by trauma or disease immediately initiates an inflammatory response, with neutrophils and macrophages migrating to the damaged muscle tissue (Fig. 3) [45,46].

Muscle regeneration for repairing damaged muscle tissue is orchestrated through several well-defined stages [47]. Muscle injury triggers an inflammatory response where immune cells such as macrophages and neutrophils migrate to the injury site to clear debris and secrete

Bone regeneration process
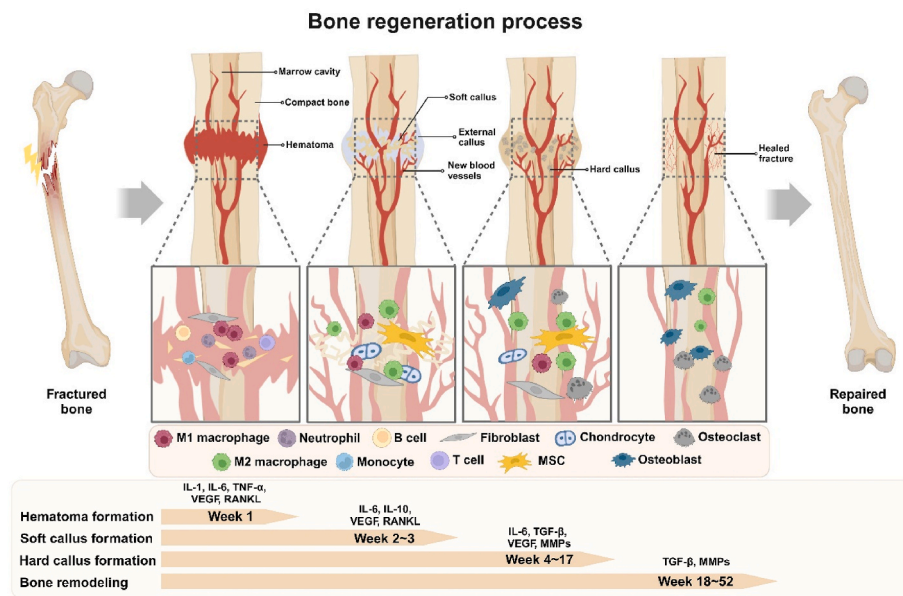
Fig. 2. Bone regeneration is a finely choreographed process unfolding over distinct phases. The initial step (week 1) involves hematoma formation, where the coagulation cascade triggers pro- and anti-inflammatory events orchestrated by IL-1, IL-6, TNF-α, VEGF, and RANKL, engaging M1 and M2 macrophages, Th1 and Th2 cells, and fibroblasts. During weeks 2–3, soft callus formation with angiogenesis is observed, driven by IL-6, TNF-α, VEGF, and RANKL, involving endothelial cells, hypertrophic chondrocytes, and osteoblasts. During weeks 4–17, hard callus formation is observed, marked by matrix mineralization and woven bone development facilitated by IL-6, TGF-β, VEGF, and MMPs, with the participation of endothelial cells, osteocytes, osteoblasts, and osteoclasts. The subsequent phase, spanning weeks 18–52, encompasses bone remodeling with TGF-β and MMPs influencing endothelial cells, osteocytes, osteoblasts, and osteoclasts, refining the healed fracture and ultimately restoring functional bone structure. This intricate temporal and cellular orchestration is fundamental for bone tissue's successful regeneration. The figure presented here is based on Y. Niu et al.'s work [42].

cytokines like IL-6 and TNF-α [48]. This sets the stage for the regeneration phase, where satellite cells—quiescent muscle stem cells located near muscle fibers—become activated. These cells proliferate and differentiate into myoblasts under the influence of growth factors and regulatory proteins such as MyoD and myogenin [49]. Myoblasts then fuse to form new muscle fibers or repair existing ones, with the extracellular matrix providing a scaffold to support tissue restructuring [50]. Finally, during remodeling, these fibers mature and the restoration of neuromuscular junctions are restored to regain muscle functionality, and blood vessels and nerves connect with the regenerated muscle fibers

Muscle regeneration process
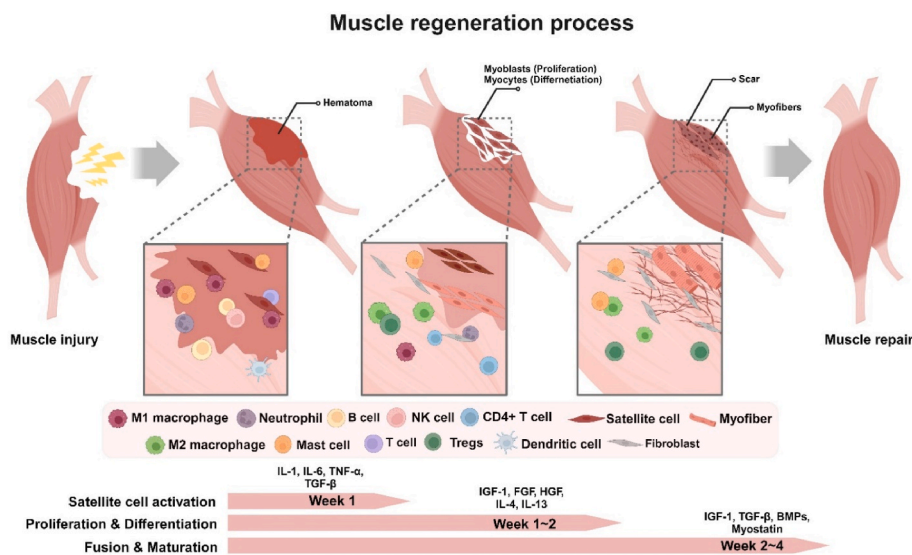
Fig. 3. Muscle regeneration is a complex and carefully controlled process that involves different phases, each with its own set of contributing cells and specific timing. The first phase (week 1) is the activation of satellite cells, which involves the formation of hematoma and initiating pro-inflammatory and anti-inflammatory events by cytokines such as IL-1, IL-6, TNF-α, and TGF-β. This phase requires the participation of various cells, such as satellite cells, macrophages, mast cells, neutrophils, eosinophils, natural killer (NK) cells, dendritic cells, and B cells. The second phase, which occurs within weeks 1–2, involves the proliferation and differentiation of myoblasts, which then transition into myocytes. This phase is influenced by factors such as IGF-1, FGF, HGF, IL-4, and IL-13 and requires the active involvement of macrophages, mast cells, T cells, Treg cells, and fibroblasts. The third phase, spanning weeks 2–4, is characterized by fusion and maturation, which involves the deposition of extracellular matrix (ECM), scar tissue formation, and myofiber development. This stage is regulated by IGF-1, TGF-β, BMPs, and myostatin, with mast cells and fibroblasts playing critical roles. Together, these steps ensure the effective regeneration and restoration of muscle tissue integrity. The figure presented here is based on the work of Muire et al. [45].

[51]. Throughout these phases, the immune system is crucial in clearing debris, combating infection, and modulating the regenerative environment to optimize healing. The high adaptability and regenerative potential of skeletal muscle can compensate for up to 20 % loss of muscle mass [52]. However, beyond this threshold, functional impairment becomes inevitable, leading to severe disability and cosmetic deformities [53].

Owing to their unique characteristics, bone and muscle tissue regeneration vary widely; however, the ultimate goal is to restore the original functionality by regenerating the tissues [54]. Advanced understanding of these mechanisms has opened up new avenues for enhancing bone and muscle repair through targeted therapies, including tissue engineering and immunomodulation, aiming to improve outcomes for individuals suffering from musculoskeletal diseases.

3. Cell therapy for musculoskeletal diseases

Regenerative medicine involves the infusion or replacement of regenerating human cells, tissues, and organs to restore their original functions. Stem cell therapy is crucial for regenerative medicine, enabling tissue recovery that was previously considered impossible [55]. Stem cells treat various diseases and damaged tissues by exploiting their unique properties with or without incorporating external genes [56]. These cells can be obtained from both embryonic and adult tissue types. Stem cells can be autologous and allogeneic. Various types of stem cells, such as embryonic (ESCs), induced pluripotent (iPSCs) [57], MSCs [58], and ADSCs [59], are employed in tissue engineering and regenerative medicine. All stem cells can differentiate into different cell types found in the body [60]. Different MSCs, especially ADSCs, are widely used for treating bone and musculoskeletal conditions. These cells can differentiate into various cell types, including bone, cartilage, and muscle cells, and have demonstrated promising results in preclinical and clinical studies. Importantly, they do not pose as many ethical concerns as those for ESCs and iPSCs, which are controversial [61].

Systemic factors from diseases can considerably affect the outcomes of cell therapy, compromising stem cell function and regenerative potential. Genomic instability [62], telomere attrition, and epigenetic changes can change stem cell behavior [63]. Conditions like chronic inflammation and metabolic diseases induce oxidative stress [64], and DNA methylation changes also cause abnormal behavior of stem cells. Environmental factors, such as deregulation of nutrient sensing pathways in obesity and metabolic syndromes, also hinder tissue regeneration [65]. Understanding these impacts is crucial for developing effective regenerative therapies and mitigating adverse effects [66].

MSCs are multipotent stromal cells of mesodermal and neural crest origin [56]. They are self-renewing and can be isolated from various tissues, including cartilage, fat, fetal tissue, muscle, and skin. MSCs regulate pluripotent differentiation potential and immune response [67]. ADSCs are available from the adipose tissue and possess several of the same regenerative properties as MSCs [68]. ADSCs are a valuable source of stem cells with self-renewing and pluripotent properties and are used to treat diabetes and autoimmune diseases. Unlike the other stem cell types, ADSCs can be easily isolated from adipose tissue, making them readily available in large quantities [69]. Moreover, unlike embryonic stem cells, no ethical concerns are involved [70]. ADSCs can also differentiate into adipogenic, osteogenic, muscle, and other tissues. Thus, using ADSCs for tissue regeneration is promising for regenerative medicine and is therefore being extensively researched [71].

3.1. Cell therapy for bone regeneration

MSCs used for cell therapy can modulate the inflammatory response by producing various growth and immunosuppressive factors such as cytokines and chemokines [72]. The differentiation is initiated by certain soluble factors in the microenvironment [73]. These factors are crucial for promoting various mechanisms that help regenerate the extracellular matrix, thereby preventing inflammation and promoting cellular proliferation, differentiation, and angiogenesis [74]. MSCs can be used to treat bone diseases and fractures. Additionally, MSCs are readily obtained from other tissues and can differentiate into cell lineages related to bone formation. MSC injections secrete biologically active molecules that regenerate damaged tissue by promoting angiogenesis and tissue regeneration and inhibiting fibrosis, cell death, and inflammation.

Stem cell differentiation is a complex interplay of various intrinsic and extrinsic factors that determine the lineage-specific outcomes after injection. For bone regeneration, various key triggers and circumstances guide stem cells to differentiate into either osteoblasts or chondrocytes. Growth factors and cytokines have crucial roles in stem cell differentiation. For osteogenic differentiation, bone morphogenetic proteins (BMPs), particularly BMP-2 and BMP-7, are pivotal in directing MSCs toward the osteoblast lineage. These factors bind to receptors on the stem cells, activating signaling pathways such as SMAD and MAPK, which upregulate osteogenic markers like Runx2 and Osterix [75]. Similarly, TGF-β and IGF are key promoters for chondrogenic differentiation. TGF-β, in particular, activates SMAD2/3 signaling, leading to the expression of Sox9, a critical transcription factor for chondrogenesis [76].

Mechanical stimuli can significantly influence stem cell differentiation. Mechanical loadings, such as compressive forces and fluid shear stress, stimulate osteogenic differentiation, activating mechanotransduction pathways, including integrin signaling and the Wnt/β-catenin pathway, promoting osteoblast differentiation [77]. In contrast, hypoxic conditions and cyclic compressive forces mimic the natural environment of the cartilage, supporting chondrogenic differentiation by enhancing the expression of cartilage-specific extracellular matrix proteins like collagen II and aggrecan [78]. These molecules could have important therapeutic applications.

The composition of the ECM is another determining factor. A stiff ECM with high mineral content, resembling the bone matrix, encourages MSCs to differentiate into osteoblasts. Scaffolds designed with hydroxyapatite or calcium phosphate can provide these osteoinductive cues. Alternatively, a softer, more pliable ECM rich in collagen and proteoglycans supports chondrogenic differentiation. Hydrogels and other biomaterials that mimic cartilage matrix are often used to guide this process [79].

Microenvironment conditions further direct stem cell differentiation. The presence of VEGF and a higher oxygen tension in the microenvironment favor osteogenesis, enhancing the recruitment and differentiation of osteoprogenitor cells [80]. Conversely, hypoxic conditions (low oxygen tension) are favorable for chondrogenesis, as they mimic the natural avascular environment of the cartilage [81].

A thorough understanding of the complex interplay of these conditions can help direct the differentiation of stem cells into specific lineages after injection, thereby enhancing the efficacy of regenerative therapies for bone and cartilage repair.

Cell therapy can be classified as autologous transplantation, in which the host cells are transplanted after in vitro culture, and allogeneic transplantation, in which cells obtained from a genetically compatible donor are transplanted. Autologous MSC treatment is patient-specific, has a low immune rejection rate, and can be administered repeatedly. Joswig et al. [82] clinically evaluated repeated intra-articular injections of allogeneic versus autologous MSCs. After the second injection, the intra-articular injection of allogeneic MSCs caused side effects such as increased lameness and synovial cell infiltration, indicating an adaptive immune response that was not observed in autologous MSCs. Therefore, repeated intra-articular injections of allogeneic MSCs may produce adverse clinical reactions, leading to immune rejection.

Allogeneic tissue transplantation is costly and require large amounts of tissue must be obtained from the host. Horwitz et al. examined bone formation after allogeneic MSC injection in children with osteogenic imperfecta. Five patients showed engraftment at multiple sites and an

accelerated growth rate for 6 months. No side effects were observed, except a rash of urticaria in one patient immediately following the second injection. Therefore, allogeneic MSCs promote osteogenesis of genetically defective bones and are safe therapeutic agents. However, allogeneic MSCs can cause immune rejection. Huang et al. [83] demonstrated that allogeneic MSCs administered via systemic or local injection did not induce an immune response while promoting osteogenesis in rats. Systemically and locally infused allogeneic MSCs promoted fracture healing without any side effects, which has clinical implications in tissue regeneration. Swart et al. [84] administered nine doses of allogeneic MSCs to a patient with systemic onset of juvenile idiopathic arthritis, and no acute infusion reactions were observed. Four of the six patients showed joint reduction 8 weeks after the first MSC administration, demonstrating that MSC injection is safe for patients with refractory juvenile idiopathic arthritis. Stempeucel® was developed for treating knee osteoarthritis using MSC derived from adult bone marrow. Gupta et al. [85] determined its safety during intra-articular administration in a phase 2 clinical trial. Allogeneic MSCs showed safety and high therapeutic efficacy in patients with osteoarthritis by differentiating into chondrogenic lineages. Therefore, allogeneic MSC injection effectively and safely induces bone formation.

Both autologous and allogeneic MSCs have distinct advantages and disadvantages, and their therapeutic applications remain highly debated [86]. MSCs must be designed for clinical applications to maximize therapeutic effects while minimizing potential side effects. Determining when to inject the stem cells for effective treatment is crucial. To identify the optimal time of injection, Wang et al. [87] monitored stem cell infusion at different time points following the fracture. MSC injection on day 7 after fracture accelerated regeneration by forming more cross-bones between the ends of the fractured bone. Moreover, they confirmed the strength and functional recovery, the core indices of bone healing, by assessing the mechanical properties (maximum load, stiffness, etc.), which improved when MSCs were injected 1 or 7 days after fracture. Thus, bone density, bone quality, and healing ability vary depending on the time of MSC injection after fracture.

Zheng et al. [88] observed that ADSCs modulated the viability of T cells to affect bone remodeling when introduced into patients with osteoporosis. Moreover, ADSCs obtained from donors with osteoporosis showed better proliferation, differentiation, and more effective bone regeneration. Contrastingly, MSCs are affected by the surrounding environment and are less productive than ADSCs in maintaining bone homeostasis.

Freitas et al. [89] injected MSCs and ADSCs into skeletal bone tissue defects in rats and evaluated bone formation. Four weeks after cell injection, MSCs induced more bone formation compared to ADSCs; both cells showed substantial bone formation rates compared to the control group. Furthermore, the mechanical properties of the newly formed bone tissue treated with MSCs and ADSCs were similar to those of existing bone. However, gene expression analysis confirmed that the bone tissue formed with ADSCs resembled the natural bone. Therefore, while ADSCs showed a slower rate of bone formation than MSCs, the bone tissue formed was nearly identical to natural bone, suggesting that ADSCs may be more advantageous for long-term bone formation.

3.2. Cell therapy for muscle regeneration

Satellite cell [90] play an essential role in muscle regeneration and remodeling. Most satellite cells are quiescent; however, they are activated during muscle damage, leading to proliferation, differentiation, and muscle regeneration [91]. MSCs can stimulate the proliferation and differentiation of skeletal muscle stem cells or satellite cells, which can help regenerate and repair damaged skeletal muscles [92]. MSCs secrete bioactive factors, including growth factors and cytokines, creating a favorable microenvironment for stem cell activity. Hepatocyte growth factor (HGF) activates c-Met signaling in satellite cells [93], thereby promoting their proliferation. MSCs release exosomes containing

microRNAs and proteins that can influence satellite cell's behavior by enhancing myogenic differentiation pathways [94], such as the Wnt/β-catenin and Notch signaling pathways. The Wnt/β-catenin pathway is crucial for regulating the balance between stem cell proliferation and differentiation, with its activation promoting myogenic differentiation by upregulating the expression of myogenic regulatory factors like MyoD and myogenin [95]. Similarly, the Notch signaling pathway helps maintain the stem cell pool by preventing premature differentiation. MSC-derived exosomes deliver Notch ligands to stem cells, facilitating some stem cells to maintain their undifferentiated state while allowing others to differentiate under specific cues [96]. Furthermore, MSCs can modulate the local immune response, which is crucial for reducing inflammation and creating a regenerative microenvironment that supports stem cell function and muscle repair. For example, MSCs can release anti-inflammatory cytokines such as IL-10 and TGF-β, which helps attenuate inflammatory response and promotes tissue healing [97]. Therefore, MSCs play a significant role in muscle regeneration-related treatments, offering a promising approach to combating inflammation and muscle damage.

MSC infusion promoted skeletal muscle healing in rats with severe muscle injury by modulating inflammatory cytokines and inducing myofibers and angiogenesis [98,99]. MSC injection following muscle damage promotes satellite cells activity, which leads to the formation of muscle fiber tissue and enables rapid muscle regeneration and good-quality muscle tissue production. Linard et al. [100] examined the quality of the muscles regenerated after MSC injection following skin and muscle tissue injury in minipigs. Without MSC infusion, the scar tissue was permanently retained in the muscle tissue, resulting in reduced muscle function. However, MSC injection accelerated macrophage migration, promoting angiogenesis and stem cell differentiation and improved muscle regeneration one year after the treatment. Andrade et al. [68] investigated whether injecting allogeneic MSCs into damaged muscles improves muscle regeneration in a mouse model. The MSCs differentiated into more mature muscle fibers with improved muscle function 28 days after forced injury. MSC injection into tight muscles did not cause fibrosis or scar formation in the damaged area, proving that allogeneic MSC injections promote regeneration and increase muscle function even in severely damaged muscles. Thus, injecting MSCs can induce immune response, inhibit inflammatory cytokines, and improve stem cell activity, resulting in more effective muscle formation and advanced muscles.

The timing of administering the MSC injection is crucial for increasing stem cell activity and promoting recovery during muscle healing. Helal et al. [99] injected MSCs into female rats 7 days after muscle injury, confirming skeletal muscle formation. MSCs were transplanted one week after muscle damage because the regenerative effect could not be perceived when the MSCs were administered immediately after injury. Without MSC injection, the density of skeletal muscles decreased at day 28 post-injury, whereas within the muscle fibers, the density of CD34 cells and capillaries increased following MSC transplantation. Additionally, satellite cell activation promoted the regeneration of damaged fibrous tissues, indicating the regeneration and maturation of muscle fibers. Winkler et al. used autologous MSCs to determine the relationship between muscle function and the number of MSCs required for muscle regeneration in rats. After injecting 0.1×10^6, 1×10^6, 2.5×10^6, and 10×10^6 cells, muscle contractility was measured; muscle strength remarkably upon transplanting 10×10^6 cells. Therefore, the appropriate amount of MSCs must be selected at the correct injection time for optimal therapeutic effect.

Satellite cell differentiation into muscle cells affects muscle regeneration and repair but requires additional treatment due to high heterogeneity and loss of differentiation. Sassoli et al. [101] postulated that using MSCs alone cannot activate satellite cell s or promote myoblast proliferation and differentiation unless combined with platelet-rich plasma (PRP). PRP aids the viability, proliferation, and differentiation of C2C12 myoblasts, thereby improving the proliferation of C2C12 cells.

Additionally, PRP activates AKT-mediated signaling, promoting the survival, proliferation, and differentiation of myoblasts, and muscle formation. The effect of PRP/MSC combination therapy is maximized by interactions between the factors contained in PRP and those released by MSC. However, further investigation is required for using MSCs alone in cell-based therapy to promote myofibroblast genesis, using PRP in non-standardized preparation procedures, and factors such as dosage, optimal dosing timing, and frequency.

Finally, the transcriptional profile of stem cells are age-dependent. Stem cells are predominantly responsible for adult muscle regeneration; however, myoblast production reduces substantially with age. Therefore, further research on promoting the proliferation and differentiation of MSCs is necessary for effectively regenerating aging muscles. ADSCs are highly effective in muscle fiber formation and regeneration. These cells possess unique regenerative properties that enable them to differentiate into muscle cells and fuse with the existing muscle fibers, leading to the growth and repair of damaged muscle tissue. ADSCs have shown remarkable potential in treating muscle injuries and disorders compared to other stem cells, making them a promising candidate for future regenerative therapies. Upon comparing MSCs and ADSCs, De La Garza-Rodea et al. found that ADSCs were the more effective cell type for myofiber formation and regeneration [102,103]. After injecting these cells into mice with skeletal injury, they compared the number of myofibers. They discovered that compared to MSCs, ADSCs induced a considerably higher degree of myofiber formation without perceptible variation in the previously observed myofiber morphology. This indicates that ADSC injection accelerates the repair of damaged muscles and does not alter the existing muscle morphology. Moussa et al. [70] compared the effectiveness of MSCs and ADSCs in promoting muscle regeneration following skeletal muscle laceration injuries. MSC- and ADSC-treated rats exhibited muscle fiber regeneration that lasted up to 8 weeks after treatment; however, MSC treatment increased collagen fibers. Collagen fiber overproduction causes loss of muscle function, which can hinder muscle recovery. However, ADSC treatment substantially reduced collagen deposition compared with that observed for MSC treatment, which may produce a higher regenerative effect. Therefore, ADSC treatment is more effective than MSC, showing higher cell proliferation capacity and accelerated muscle recovery. ADSCs are extensively used in cell therapy clinical trials; however, the efficacy of ADSCs remains inconsistent compared with that of MSCs [71]. Moreover, the physiological mechanisms underlying their clinical use are not entirely understood; therefore, local and systemic delivery mechanisms for ADSC infusion require further investigation [104]. Stem cell-mediated tissue regeneration has many therapeutic benefits, such as reduced side effects using autologous tissue. However, some limitations exist. The number of obtainable stem cells decreases with age, and the injected stem cells are not retained in the body for long periods. Additionally, donated stem cells may cause immune rejection, leading to complications. Autologous transplantation is safer; however, large quantities of these cells are difficult to obtain. Although preclinical models show therapeutic.

efficacy and results from clinical studies are unremarkable [105]. Data on the long-term effects of cell transplantation remain insufficient, and various exogenous substitutes have been introduced and used in clinical trials to overcome these limitations of autologous transplantation.

3.3. Modulating the immune system using cell therapy

Due to their low immunogenicity, MSCs can evade the immune system. This property enables them to be transplanted into patients without eliciting a strong immune reaction, a feature especially beneficial for allogeneic transplantation across different individuals. This attribute is particularly vital in treating musculoskeletal disorders, in which the pro-inflammatory environment impedes healing. MSCs accomplish immunomodulation primarily via four ways. Firstly, MSCs produce a range of immunosuppressive factors, notably anti-inflammatory cytokines such as TGF-β and IL-10 [106]. These cytokines suppress the activation and proliferation of various immune cells, including T cells, B cells, and natural killer (NK) cells, thus mitigating the potential for immune rejection. Secondly, MSCs induce regulatory cells, especially facilitating the development of regulatory T cells, a critical subset of T cells responsible for maintaining immune tolerance. By promoting regulatory T cells in number and function, MSCs foster a more tolerant local immune milieu, diminishing inflammation and enhancing tissue repair. miRNAs such as miR-182 or miR-223, abundant in MSC extracellular vesicles, act as positive regulators of anti-inflammatory pathways [107,108]. Thirdly, MSCs influence the maturation and function of dendritic cells, which are antigen-presenting cells that can activate T cells and spark immune responses. MSCs can render dendritic cells into a state less likely to provoke a robust immune reaction against the introduced cells or tissues, thereby aiding in the acceptance and integration of therapeutic agents. Fourth, MSCs can shift macrophages from a pro-inflammatory M1 phenotype towards a more anti-inflammatory M2 phenotype. M2 macrophages contribute to tissue repair and regeneration, releasing factors promoting healing and attenuating inflammation. Proteins such as MSC-produced CCR2 act as a decoy to bind and reduce free extracellular levels, and the chemokine *C*–C motif ligand 2 functions [109]. Incorporating these mechanisms into the context of tissue engineering and regenerative medicine underlines the significance of MSCs in creating a conducive environment for the repair and regeneration of musculoskeletal tissues. Their immunomodulatory capabilities are pivotal for reducing the likelihood of immune rejection and enhancing the effectiveness of regenerative therapies. This transformation, as illustrated in Fig. 4, showcases the immunomodulatory potential of cell therapy, reducing inflammation and enhancing tissue repair in musculoskeletal diseases.

3.4. Clinical trials of cell therapy

Recently, numerous clinical trials have examined the efficacy of cell therapy for various medical conditions, a few of which are summarized in Table 2. For example, a trial investigating MSCs for knee osteoarthritis demonstrated the efficacy of autologous MSC injections in alleviating persistent pain [110]. MSCs, injected into the joint space, exert immunomodulatory effects to reduce inflammation and promote cartilage repair. Unlike traditional immunosuppressive therapies that broadly reduce immune function, MSCs targeted specific immune responses in the joint, preventing the immune system from attacking the introduced cells and allowing them to integrate and repair tissue. Another study focused on using ADSCs for avascular necrosis of the femoral head. However, the lesion size showed no significant reduction and side effects data were unavailable (NCT01643655). Conversely, an ADSC trial for knee osteoarthritis reported improvements with autologous adipose tissue-derived MSC injections, although some patients experienced side effects such as joint pain and soreness (NCT02674399). Another MSC trial for avascular necrosis of the femoral head showed positive outcomes, with osteogenesis observed in most patients [111]. An MSC trial for lateral epicondylitis reported a significant reduction in defect area after 12 weeks (NCT01856140). Recent trials include a study on MSCs for Duchenne muscular dystrophy, indicating no dose-limiting toxicity and favorable effects on muscle cell death, fibrosis reduction, and muscle regeneration (NCT05338099). Additionally, a trial exploring MSCs for osteoarthritis found comparable improvement in knees treated with cell-based or corticosteroid injections (NCT03818737). These studies highlight the promise and challenges of using stem cells to treat complex disorders such as osteoarthritis, avascular necrosis, and muscular dystrophies. Therefore, while these trials provide promising evidence supporting the use of stem cells in regenerative medicine, they also highlight the challenges and complexities involved. Future research should focus on optimizing cell preparation methods, understanding patient-specific responses, managing side
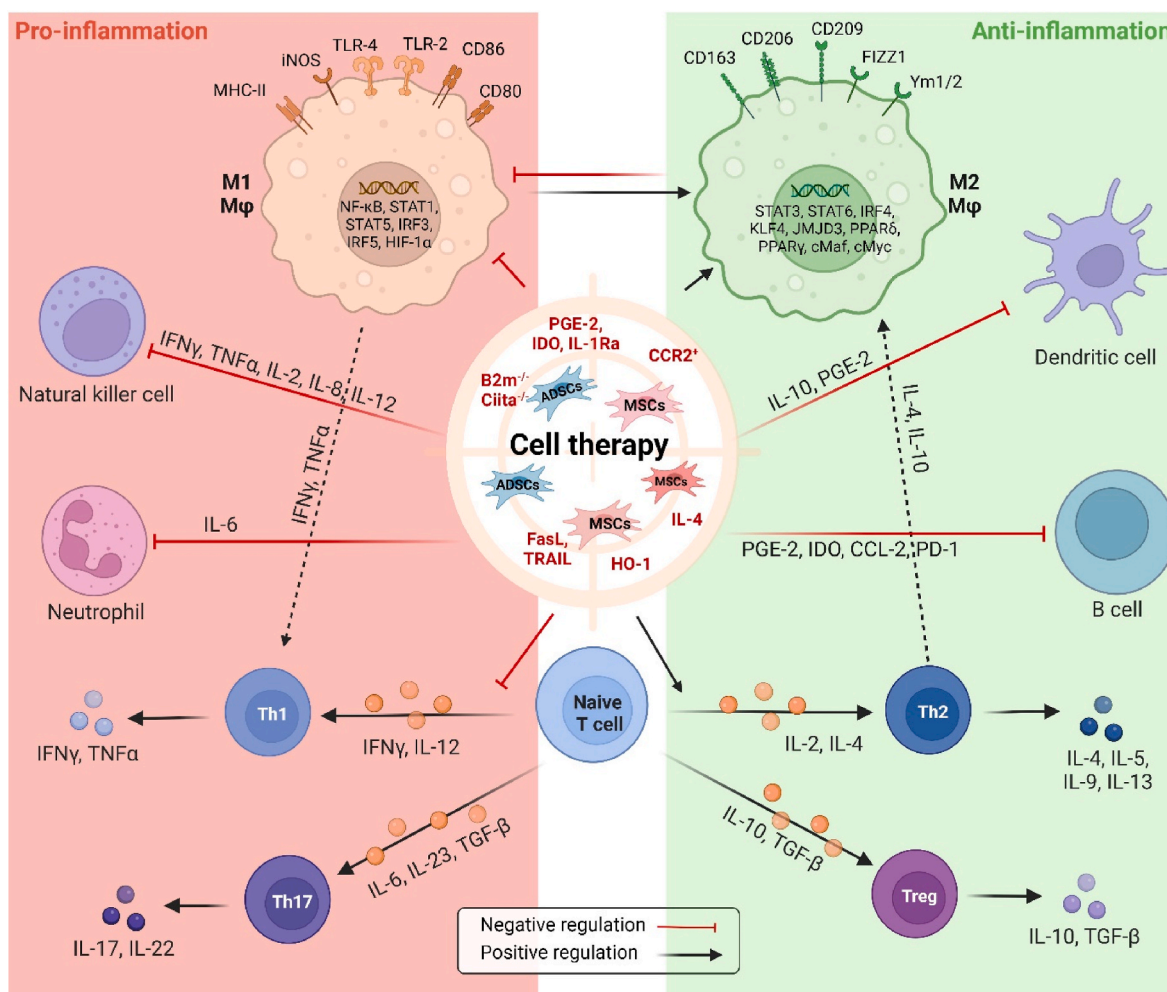
Fig. 4. Tissue engineering and regenerative medicine can be used to evade the immune system and harness its features for therapeutic purposes. Mesenchymal stem cells (MSCs) can potentially reduce the damage caused by the immune system. The biomolecules secreted by MSCs can cause a transition of M1-like cells to M2-like macrophages, which significantly promotes anti-inflammation and reduces inflammation. This is achieved by down-regulating Th1 and Th17 cells while positively stimulating T regs and Th2 cells. MSCs can influence the immune system by releasing specific factors that regulate various immune cells. The release of IFNγ, TNFα, IL-2, IL-8, and IL-12 inhibits natural killer (NK) cells. B cells are regulated through PGE-2, IDO, CCL-2, and PD-1. IL-10 and PGE-2 control dendritic cells, while IL-6 regulates neutrophils. This well-coordinated interplay highlights the potential of MSCs to fine-tune immune responses and offers promising therapeutic options for tissue engineering and regenerative medicine.

Table 2
Summary of recent clinical trials of stem cell therapies.

Tissue	Cells	Conditions	Outcomes	Completed	Identifier
Bone	MSCs	Osteoarthritis, Knee, Knee Degenerative Disease, Knee Osteoarthritis	- Autologous MSC injection improves disease by relieving persistent pain. - Disease improvement is clear, but the cell preparation is expensive.	September 2014	NCT01183728
	MSCs	Avascular Necrosis of the Femoral Head	-No significant side effects, but no reduction in lesion size.	Mar 2015	NCT01643655
	MSCs	Avascular Necrosis of the Femoral Head	-Osteogenesis was seen in 16 of the 22 patients, with the head retaining its spherical shape.	December 2017	NCT02065167
	ADSCs	Osteoarthritis, Knee	-Results showed improvement when autologous adipose tissue-derived mesenchymal stem cells (Jointstem) were injected. Side effects of joint pain and soreness have also been observed.	December 2018	NCT02674399
	MSCs	Osteoarthritis	- MSC injections into one knee and saline injections into the other knee show the same improvement in both knees. -Cell-based injections are no more effective than corticosteroid injections.	May 2022	NCT03818737
Muscle	ADSCs	Lateral Epicondylitis	-Significant reduction in defect area after 12 weeks.	April 2018	NCT01856140
	MSCs	Duchenne Muscular Dystrophy	-Low and high doses show no dose-limiting toxicity, with inhibition of muscle cell death, reduction of muscle fibrosis, and muscle regeneration.	December 2022	NCT05338099

Table 3
Summary of recent clinical trials of biomaterials.

Material	Condition	Description	Completed	Identifier
Beta-TCP/bovine collagen matrix, rhpdgf-BB	Degenerative Joint Disease, Congenital Deformity, Arthritis, Osteoarthritis, Rheumatoid Arthritis	- The mean fusion time was 14.3 ± 8.9 weeks for patients with support implants and 19.7 ± 11.5 weeks for patients with autografts. - After 24 weeks, complete fusion of all joints was seen in 53 of 64 patients with support implantation and 100 of 154 with autograft treatment, indicating faster healing with support implantation. - Painless when implanted and promotes bone formation, effectively indicating bone fusion.	April 2014	NCT01305356
Small intestinal submucosa (SIS), urinary bladder matrix (UBM), dermal ECM-derived scaffold	Traumatic Injury, Muscle Injury, Tendon Injury, Soft Tissue Injury, Extremity Injury	- After 6 months, β-III Tubulin + cells were increased in the area where the scaffold was implanted, indicating the formation of innervated skeletal muscle. - Thirteen patients implanted with cell-free ECM scaffolds demonstrated increased soft tissue formation consistent with skeletal muscle tissue.	May 2015	NCT01292876
Osteostrux™ Collagen Ceramic Scaffold	Degenerative Changes, Stenosis, Spondylosis	- Combined with bone marrow aspirate, OsteoStrux Strips are currently used in orthopedics to effectively absorb into voids or gaps where bone is present and replace it with bone.	October 2016	NCT01873586
Equimatrix®, bio-oss®, endobon®	Alveolar Bone Loss	- Equimatrix is better when comparing new bone formation and bone density after implantation.	December 2016	NCT03149172
Tailored amorphous multiparous bioactive glass	Bone Loss, Vertical Alveolar Bone Loss, Horizontal Alveolar Bone Loss	- TMAP scaffold-grafted 14 % of sockets achieved 50 % average bone density after 3 months, compared to none of the control sockets. - At week 15, the trabecular arrangement appeared denser and sculptural, mainly in the vertical direction. - Enhanced stem cell recruitment in the scaffold-grafted sockets, promoting active bone modeling.	January 2018	NCT01878084
Bone mineral (ABM), P-15	Intervertebral Disk Degeneration	- Fusion rates comparable to autologous bone graft rates.	May 2019	NCT00310440

effects, and comparing new therapies with existing standards. By addressing these challenges, the full potential of cell therapy in treating musculoskeletal disorders could be realized (see Table 3).

4. Bioactive materials for musculoskeletal diseases

Biomaterials are engineered substances typically used for medical purposes that interact with biological systems. Their functionality is attributed to well-defined properties, broadly categorized into mechanical, physical, and biocompatibility characteristics, which determine their suitability and performance in clinical applications. Mechanical properties dictate how biomaterials behave under the various stresses they encounter within the body. These include hydrostatic pressure, compressive forces from weight or other forms of stress, and specific localization of mechanical forces. Fluid shear, the stress exerted by fluid flow, vibration, tension, and shear forces, also significantly influences the resilience and longevity of the material in dynamic biological environments (Fig. 5a). Physical properties of biomaterials include structural and textural features, pivotal for their integration into biological systems. This encompasses compartmentalization, which defines the spatial configuration of the material, and surface roughness, a factor that can affect cellular response and tissue integration. Toughness and porosity govern the ability of the material to absorb energy without fracturing and its permeability to fluids and nutrients. The pattern, shape, and size are tailored to mimic the natural architecture of tissues, enabling biomaterials to scaffold and support cellular activities (Fig. 5b). A biocompatible material can integrate favorable properties without eliciting a negative host response. This includes non-toxicity, appropriate water content for cellular interactions, osseointegration for materials interfacing with bone, and non-combustibility for safety (Fig. 5c). Chemical properties such as the pH level, charge, and hydrophilicity or hydrophobicity significantly affect protein adsorption, cell adhesion, and the overall bioactivity of the material. Moreover, functional groups and the chemical structure of the biomaterials can be designed to facilitate specific interactions with cells and biomolecules, promoting healing and regeneration (Fig. 5d).

The central depiction of various biomaterials in the image, ranging from nanoparticles to fibrous scaffolds and hydrogels, signifies the diverse applications of these materials. Whether used for bone regeneration, wound healing, or as carriers for drug delivery, each biomaterial is engineered with a precise combination of these mechanical, physical, and chemical properties to optimize its interaction with the human body. The intricate balance of these properties make biomaterials indispensable in advancing regenerative medicine, prosthetics, and therapeutic delivery, ultimately enhancing patient care and medical outcomes.

4.1. Importance of intermingling of biomaterials-cell therapy

Bioactive materials are used for tissue regeneration and repair to overcome the limitations of cell-based regenerative medicine treatments [112]. Bioactive materials are used with living cells, biomolecules, physical factors, external stimulations, or a combination to create tissue-like structures to replace damaged tissues or organs. Biomaterials play a crucial role in regenerative medicine, acting as a carrier for cells and providing a favorable environment for their growth and development. These materials can be engineered to mimic the natural extracellular matrix, allowing for enhanced cell attachment, proliferation, and differentiation. Moreover, researchers can create a customized microenvironment that promotes tissue regeneration and repair by selecting the appropriate biomaterial and tailoring its properties [113]. Recently, the physical characteristics of biomaterials have been modified before employing them in mechanotherapy. This technique uses vibration, tension, pressure, compression, and shear. Mechano-therapy can potentially help in the development of innovative treatment methods that can enhance the outcomes of tissue engineering and regenerative medicine.

4.2. Bioactive materials for bone

The materials, cells, and signaling pathways must be carefully considered before their use in tissue engineering for bone formation.
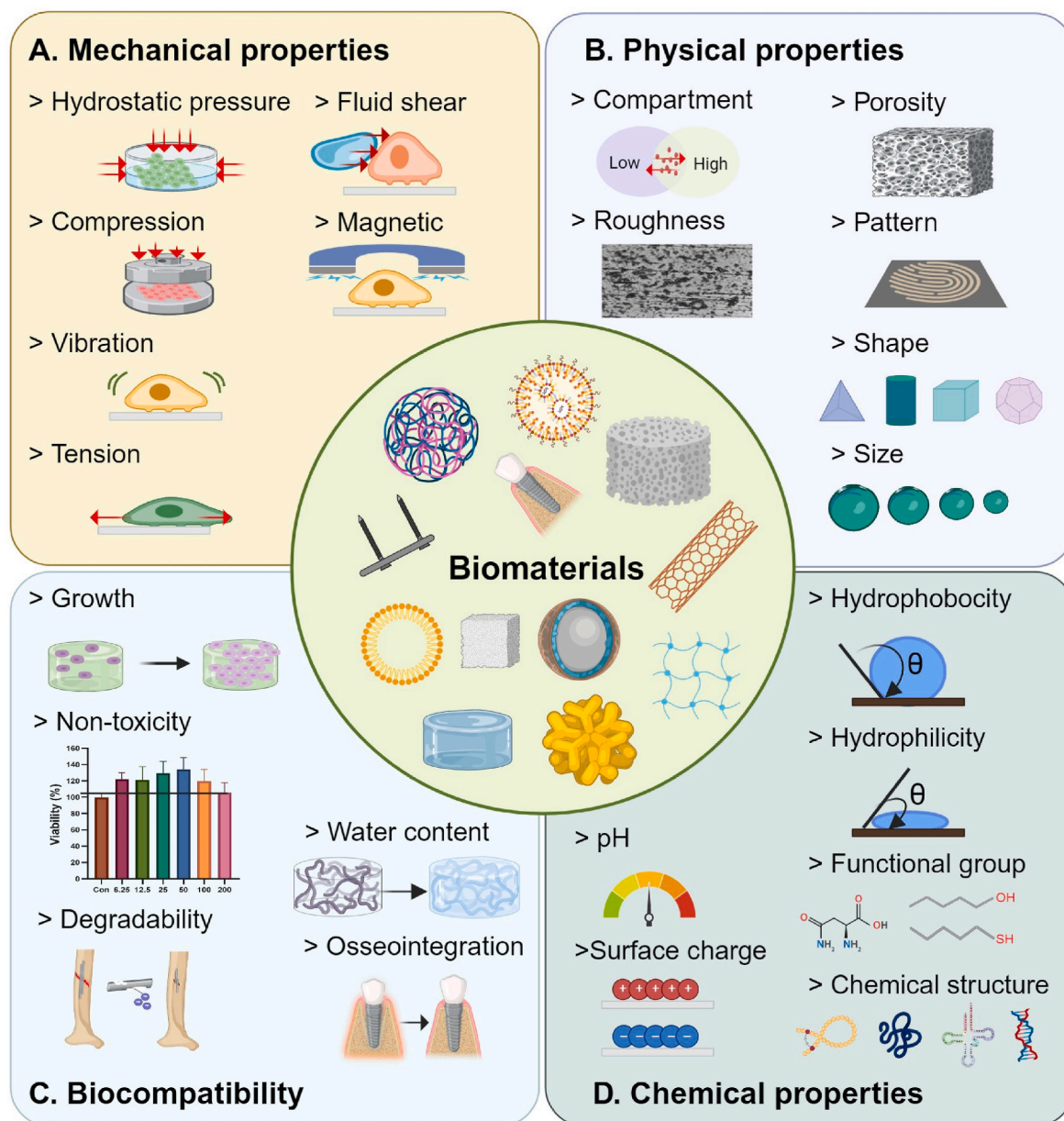
Fig. 5. Natural or synthetic biomaterials interact with biological systems to trigger, treat, or support affected areas by leveraging their diverse properties. These materials can repair or regenerate tissues through mechano-therapy, utilizing vibration, tension, and compression forces. Their porosity and patterning ensure biocompatibility by supporting cell growth and ensuring non-toxicity. Furthermore, they have specific chemical properties, including surface charge and functional groups. These characteristics influence cellular processes such as adhesion, proliferation, and migration, providing benefits beyond structural support in tissue engineering and regenerative medicine.

Stem cell types have been differentiated into osteoblast lineage cells and used for bone regeneration. Loading stem cells onto scaffolds is a promising strategy for promoting bone regeneration.

MSCs and ADSCs are the most widely used stem cells in bone regeneration clinical trials [114] in combination with biomaterials. Although bone regeneration typically involves implanting tissue from the patient or a donor, tissue shortage or donor incompatibility necessitates the fabrication of biomimetic scaffolds in bone tissue engineering [115]. An ideal scaffold should be made from a biocompatible material, which promotes tissue regeneration by regulating cell migration, proliferation, and differentiation after implantation and replaced entirely by autologous tissue [116]. Popular bone-regeneration tissue engineering techniques aim to achieve faster bone formation by adding cells and growth factors to scaffolds. The materials, cells, and signaling choices of the scaffold are critical for effectively inducing bone

formation [117]. Thus, scaffold characteristics are essential for effective tissue regeneration. Scaffolds must be biocompatible to support cell adhesion, proliferation, and differentiation without eliciting an adverse immune response. Materials like polylactic acid and polyglycolic acid are commonly used due to their excellent biocompatibility and biodegradability, which allow the scaffold to gradually disappear as new tissue forms, reducing the need for surgical removal [118]. Scaffolds must be capable of withstanding physiological loads and providing structural support to the regenerating tissue. These properties can be tailored for the required tissues by adjusting the composition and fabrication process of the scaffold [119]. The pore size, shape, and interconnectivity are crucial for facilitating cell infiltration, nutrient diffusion, and waste removal, with optimal pore sizes varying depending on the tissue regenerating type [120]. Hydrogels, such as those composed of alginate or collagen, provide a hydrated environment that

supports cell viability and proliferation. Their swelling helps maintain a moist environment conducive to cell survival and function. Additionally, incorporating bioactive molecules like growth factors (e.g., TGF-β1, BMPs [121]) or peptides can enhance the ability of the scaffold to promote specific cellular responses and guide tissue regeneration, which will be discussed in section 5. By integrating these scaffold characteristics into the design, more effective biomaterials can be created to support and enhance the regeneration of various tissues [122,123].

In acellular tissue engineering, a natural or synthetic matrix regenerates tissues using only a scaffold without any cells. The scaffold used in the damaged area tends to degrade over time and be replaced by regenerated cells and ECM produced by cells.

The material used for bone formation must be biocompatible and have properties similar to those of the bone, which comprises inorganic and organic components [124] in the following composition: 60 % inorganic components (hydroxyapatite (HAp) [$Ca_3(PO_4)_2$]3Ca(OH)$_2$), 30 % organic components (mainly proteins such as collagen), and 10 % water. Therefore, a composite scaffold fabricated with inorganic and organic components could promote bone formation without cells.

Inorganic and organic components can be combined with other materials to create scaffolds that promote bone formation. Sun et al. [125] fabricated a porous composite scaffold using human bone powder (BP) comprising HAp obtained from decellularized porcine dermal tissue. The mechanical properties were improved by adjusting the BP levels to 05 %, 25 %, and 50 %. Furthermore, increased osteogenic protein (Runx2, ALP, OPN) content and osteogenic activity were observed with increased BP. In vivo osteogenesis evaluation using a Sprague–Dawley rat skeletal model showed that a higher BP content promoted the formation of new and more denser bones with a substantially higher rate of bone regeneration.

Matta et al. showed that an aragonite-based acellular scaffold promoted bone formation. The aragonite-based scaffold comprised 98 % calcium carbonate, encouraging bone formation without cells [126]. In vitro, MSCs were exposed to the scaffold to confirm the transformation and morphology of the cells, which appeared thicker and spindle-shaped with considerable visible mineralization. The levels of osteogenic markers (RUNX2, ALP, BGLAP, SPARC, and SPP1) increased substantially in the presence of aragonite-based scaffolds, suggesting that acellular aragonite scaffolds can be used for bone remodeling.

Combinations of organic and inorganic components have been used to fabricate scaffolds that promote bone formation. Zhou et al. [127] fabricated a scaffold with a three-layer structure comprising calcium phosphate (CaP), collagen (Col), and hydroxyapatite (HAp) to mimic the composition and structure of natural bone tissue, making it resistant to deformation by increasing its mechanical strength and compression coefficient. MSCs were cultured in vitro to evaluate the biocompatibility of the CaP/Col/HAp scaffolds. The cells cultured on the scaffolds exhibited better proliferation with no observed morphological differences from those on conventional scaffolds. After implantation in rabbits, the CaP/Col/HAp scaffold was biocompatible and showed improved osteoinductivity and newly formed bone tissue was observed around it. Therefore, when fabricating acellular scaffolds for bone tissue regeneration, the materials selected must have good mechanical properties that promote biological response.

Mahon et al. [128] fabricated different ECM-derived scaffolds and implanted them into a rat model with femoral defect. The growth plate-derived ECM scaffold promoted neovascularization and maturation in rats, confirming its effectiveness in muscle regeneration. They examined the efficacy of different ECM-based scaffolds and explained that proper ECM selection influences the behavior of stem cells and macrophages for effective tissue regeneration.

4.3. Bioactive materials for muscle

Biomaterials provide a conducive environment for differentiating progenitor cells, which is critical for repairing muscle cells. Biomaterials used in muscle regeneration should create a biomimetic microenvironment to promote tissue maturation and enhance function, be biocompatible, and effectively support cell adhesion. Fabricated biomaterials include natural ECM, synthetic polymers, ceramics, metals, and composites [129]. Muscle tissue engineering requires the differentiation of skeletal muscle myoblasts or muscle precursors into multinucleated myotubes [130]. For muscle injury, tissue engineering is used primarily to repair the damage by mimicking the microenvironmental cues of existing muscle tissue [131]. Sicari et al. demonstrated that acellular biologic scaffolds composed of ECM can significantly promote muscle regeneration in preclinical rodent models and human patients with volumetric muscle loss [132]. Porcine urinary bladder ECM scaffolds were implanted at the site of muscle injury, which provided a supportive microenvironment that facilitated the mobilization and accumulation of perivascular stem cells and promoted neovascularization. The ECM scaffolds altered the default healing response from scar tissue formation to constructive tissue remodeling. These processes led to the de novo formation of skeletal muscle fibers, as evidenced by the presence of desmin-positive and myosin-heavy chain-positive cells, and resulted in functional improvements, such as enhanced muscle strength and electromyographic activity. This study highlighted the potential of ECM-based scaffolds as an effective treatment strategy for improving muscle regeneration and restoring function in patients with severe muscle injuries. Furthermore, all the engineered tissue should be able to interact with the organic body and promote regeneration while fusing with the existing tissue without causing side effects.

Basurto et al. [133] fabricated the collagen–glycosaminoglycan–-polypyrrole scaffold and confirmed muscle formation and maturation. The scaffolds had longitudinally aligned pores, which was achieved using polypyrrole. Polypyrrole enhanced the metabolic activity of myofibroblasts by increasing scaffold conductivity, which facilitated electron transfer and boosts ATP production. Polypyrrole also activated the AKT signaling pathway, improving cell metabolism and protein synthesis. Additionally, polypyrrole provided mechanical and biochemical cues that promote cell adhesion, spreading, and proliferation, further stimulating metabolic activity. Polypyrrole doping was highly effective for muscle regeneration compared to scaffolds made of collagen only.

4.4. Clinical trials of biomaterials

The number of completed clinical trials investigating the effectiveness of tissue regeneration materials is limited. One study explored using the Beta-TCP/bovine collagen matrix with rhPDGF-BB to treat various conditions, including degenerative joint disease, congenital deformity, arthritis, osteoarthritis, and rheumatoid arthritis. This trial found that implanting the matrix resulted in faster healing and complete fusion of joints, with the added benefits of being painless and promoting effective bone formation [134]. Subsequent trials provided further insights. One study focused on small intestinal submucosa, urinary bladder matrix, and dermal ECM-derived scaffold to treat traumatic, muscle, tendon, soft tissue, and extremity injuries. The findings revealed increased innervated skeletal muscle formation and soft tissue that resembled skeletal muscle tissue after 6 months [135].

Another study investigated the efficacy of Osteostrux™ Collagen ceramic scaffold combined with bone marrow aspirate to treat degenerative changes, stenosis, and spondylosis. The outcomes demonstrated that the scaffold effectively replaced bone by actively absorbing into voids or gaps (NCT01873586). A study evaluated materials such as Equimatrix®, bio-oss®, and endobon® for treating Alveolar Bone Loss. The trial concluded that Equimatrix was superior regarding new bone formation and bone density after implantation (NCT03149172). One trial explored the potential of a Tailored Amorphous Multiparous Bioactive Glass scaffold to treat bone loss and vertical and horizontal alveolar bone loss. The study showed that the scaffold enhanced stem cell recruitment and promoted an active bone modeling process, offering

promising results [136]. Lastly, a trial investigated Bone Mineral and P-15 for treating intervertebral disk degeneration, reporting fusion rates comparable to autologous bone graft rates (NCT00310440). These study results have contributed to the ongoing pursuit of effective tissue regeneration strategies with diverse materials and applications.

5. Biomolecules and external stimulation for bone and muscle regeneration

5.1. Importance of intermingling of biomaterials-biomolecules and external stimulation

The primary objective of biocompatible materials is to foster a highly supportive environment that stimulates cell growth and proliferation. These materials, with their ability to mirror the shape, pattern, size, and porosity of a cellular environment, create an ideal microenvironment that closely mimics the physical and chemical properties of the extracellular matrix. Moreover, they can release biomolecules that enhance the microenvironment, thereby facilitating cell growth and proliferation. Their unique feature of prolonged biomolecule release makes them an exceptional vehicle for delivery within the body. Additionally, biocompatible materials can respond to external stimuli such as temperature or light, and this can be leveraged to directly and efficiently enhance cell growth.

Biomolecules and external stimulations, pivotal elements in tissue engineering and regenerative medicine, are extensively used with various biomaterials. They are critical in developing new and effective treatments for different medical conditions. The interaction between a biomaterial and biomolecule, or a biomaterial and external stimulation, is a crucial factor for successfully regenerating the natural condition. Recently, growth factors, peptides, and aptamers have emerged as the most widely used biomolecules in combination with biomaterials. Similarly, electrical fields, magnetic fields, topography, ultrasound, and various light wavelengths have gained prominence as external stimulations.

5.2. Biomolecules

Growth factors are important mediators of tissue regeneration and an effective tool to induce cellular healing and regeneration [83,98]. They are proteins that stimulate cell division and differentiation and are essential in tissue repair [99]. The activation and proliferation of satellite stem cells initiate the regeneration of muscle tissue. HGF is released immediately after injury and implicated in skeletal muscle development and regeneration. Grasman et al. [137] promoted tissue regeneration in a muscle injury model after injecting HGF into a cross-linked fibrin scaffold. This led to the rapid release and activation of stem cells at the wound site, thereby increasing the number of myoblasts. Suliman et al. showed that BMP-2 delivery by scaffold attenuates inflammation, causing the delivery system to degrade slower [138]. Combining growth factors with scaffolds is a promising strategy for promoting enhanced tissue regeneration. Soluble factors like TGF-β are also used with scaffolds for tissue engineering. TONG et al. developed a three-dimensional scaffold composed TGF-β1, silk fibroin (SF), and chitosan (CS) to evaluate its potential for bone tissue engineering. The TGF-β1-SF-CS scaffolds have demonstrated their ability to enhance the viability and proliferation of bone MSCs and promote osteogenesis and their practical implications. The introduction of TGF-β1 has proven to be a key factor, significantly enhancing new bone formation and demonstrating the efficacy of the scaffold for bone tissue engineering [139].

Peptides are often used in bone regeneration research, with different types showing varying effects. BMP mimetic or capturing peptides, for example, are commonly used. BMP-2 mimetic peptides conjugated to alginate-maleimide microcapsules have been used to stimulate osteogenic differentiation [140]. Osteogenic growth peptide was found to enhance bone formation. Xu et al. utilized OGP conjugated to polypropylene fumarate to create a scaffold that can effectively bind to Bioglass, a highly bioactive material widely used in tissue engineering and regenerative medicine. They first modified the osteogenic growth peptide via PEGylation and then attached dendritic catechol groups [141]. The study showed that dual functionalization of poly (propylene fumarate) with Bioglass and bioactive peptides improves human MSC osteogenic differentiation. A combined strategy can be employed by utilizing growth factor-specific peptide and hydrogel to address cartilage regeneration challenges due to traumatic injury, excessive wear, or age-related degeneration. Traditional methods often fail to restore hyaline cartilage's mechanical properties and durability. One research group explored a novel Gelatin methacryloyl (GelMA) hydrogel modified with a TGF-β1-affinity peptide (HSNGLPL) to enhance cartilage regeneration. The peptide in the hydrogel has a high affinity for TGF-β1, thereby promoting cartilage regeneration. The study underscores the urgency of finding an alternative to exogenous TGF-β1 delivery. This method, which uses a photo-crosslinked GelMA hydrogel modified with a TGF-β1-affinity peptide, enhances cartilage regeneration by recruiting endogenous TGF-β1. By addressing the limitations associated with exogenous TGF-β1 delivery, such as the need for supraphysiological concentrations and regulatory challenges, this research paves the way for a more practical approach. The photo-crosslinked GelMA hydrogel with TGF-β1-affinity peptide demonstrates its potential by effectively recruiting endogenous TGF-β1, promoting cartilage regeneration. This novel approach is promising clinical cartilage repair applications, offering a bioactive scaffold with excellent mechanical properties and biocompatibility [142].

Aptamers are used to target specific cells in the field of bone and cartilage. Recently, particular aptamers have been developed to target human pluripotent stem cells [143]. For functionalization, scaffolds were conjugated with aptamers, which stimulated directional differentiation of MSCs in vivo and supported new tissue formation [144]. This technology was also used to target osteoblasts. Liang et al. found an aptamer called CH6, which can specifically target osteoblasts. The aptamer was linked with lipid nanoparticles that could release incorporated siRNA within the cell. This resulted in better bone formation, structure, bone mass, and mechanical properties in in-vivo experiments [145]. Recently, a cell-specific aptamer with extracellular vesicles was used to stimulate mineralization and in vivo bone regeneration [146].

Growth factors are primarily proteins with a brief shelf-life, high production cost, and marked instability compared to peptides and aptamers. Peptides and aptamers exhibit low immunogenicity, low production cost, and easy modifiability. However, compared to aptamers, peptides demonstrate less specificity towards targets. In the foreseeable future, aptamers could be employed as a highly productive tool for target-specific interventions using a composite biomaterial.

5.3. External stimulation

The multifaceted role of external stimulation in muscle and bone regeneration includes the therapeutic potential of electrical, ultrasound, topographical, magnetic field interventions, and light wavelength therapies [147]. These diverse external stimulation modalities augment intrinsic tissue regeneration and synergize with bioengineered scaffolds and cellular therapies to provide a comprehensive approach to regenerative medicine. Treatment of musculoskeletal disorders has been significantly advanced by integrating electrical [148], ultrasound [149], topographical [150], magnetic [151,152], and photonic stimulations [153] into clinical practices. These techniques aim to expand current capabilities in tissue regeneration, offering enhanced outcomes for patients with musculoskeletal injuries (Fig. 6) [154].

Electrical stimulation has garnered attention as an alternative physical method for muscle cell regeneration. Electrical stimulation activates intracellular signaling pathways that affect cell migration, proliferation, and differentiation. Electrical stimulation activates the epidermal growth factor receptor, which further regulates cell migration
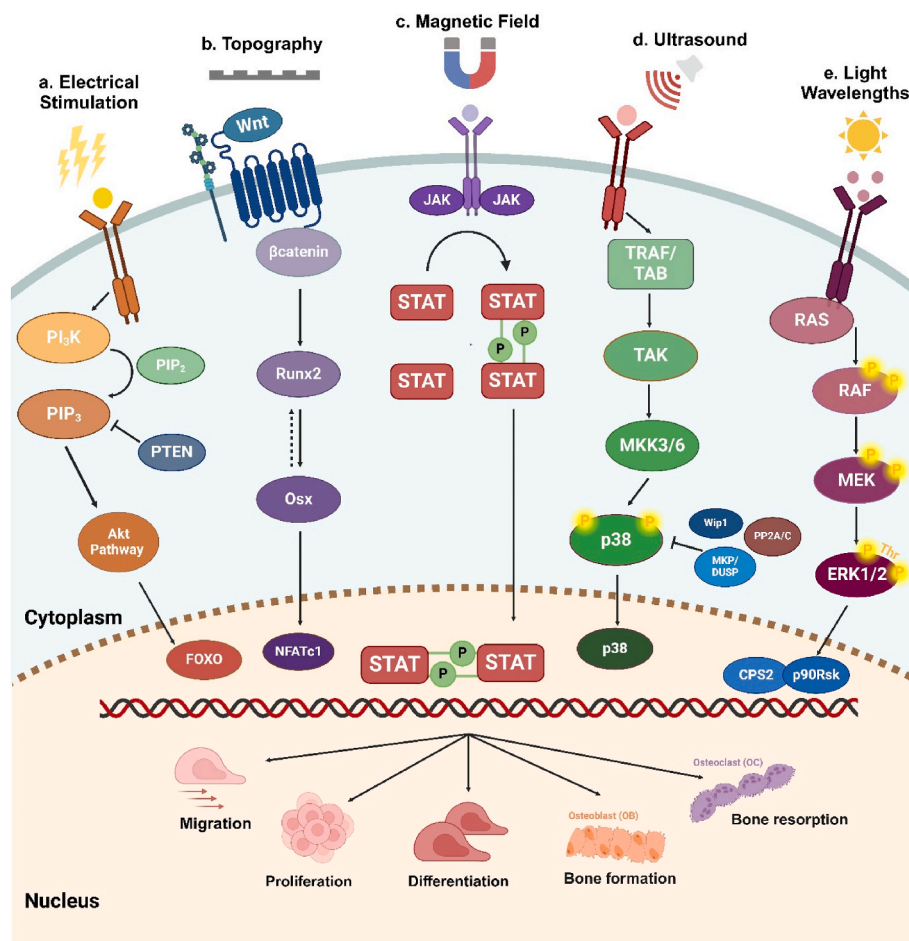
Fig. 6. External stimuli activate specific molecular pathways within cells, triggering unique molecular changes. Electrical stimulation activates the Akt pathway (a), changes in topography stimulate the Wnt pathway (b), while a magnetic field engages the JAK-STAT pathway (c). Ultrasound activates the ERK/MAPK-P38 pathway (d), and light wavelengths modulate the Ras-Raf-MEK pathway (e). These pathways control cellular behavior in response to external stimuli, promoting cell proliferation, differentiation, and migration.

through the MAPK-ERK1 [155] and PI3K-Akt [156] pathways (Fig. 6a). Additionally, ion channels are susceptible to electrical stimulation, promoting migration [157]. Parameters for electrical stimulation include the voltage type, amplitude, and frequency, which can be changed to modulate muscle morphology, differentiation, and proliferation [26]. Different osteo-inductive factors were released upon electrical stimulation, promoting synergistic bone repair [158–160]. Furthermore, muscle tissue growth was enhanced using different electrical frequencies, and improved myo-bundle maturation and contractile force were also observed, thereby mimicking muscle activity [13,76].

Topography is the most effective and preferred alignment technique for tissue regeneration. The surface roughness of materials stimulates osteoblastic differentiation of MSCs by activating multiple signaling pathways. Among these pathways, the transcription factors Runx2-Osx have emerged as critical determinants of osteogenic differentiation (Fig. 6b) [95]. Particularly for muscle cells, alignment in one direction is necessary for effective fusion. The alignment of muscle fibers substantially affected skeletal muscle structure, as grooves and widths formed more mature and aligned myotubes than when cells were grown on flat substrates [161]. Therefore, proper sizing of grooves and patterns is essential for obtaining cells with aligned morphologies in specific directions.

Static- and electro-magnetic fields can substantially enhance bone repair and regeneration. Magnetic nanoparticles, when used alone or combined with a magnetic field, are promising for bone tissue engineering applications. Magnetic nanoparticles can substantially modify

and improve the three critical factors in bone regeneration - stem cells, scaffolds, and growth factors [162]. Magnetic scaffolds can be prepared using magnetic nanoparticles and magnetic fields; a magnetic field can enhance the cells via up- and down-regulation of the PPAR signal transduction and JAK-STAT pathway, respectively (Fig. 6c) [163]. Magnetic fields can directly impact the ion channels and biochemical pathways via cell labeling, targeting, patterning, and gene modification, and they can increase the effectiveness of these magnetic fields. Magnetic field stimulation was recently found to dynamically program tissue anisotropy during skeletal muscle differentiation [164].

Ultrasound, especially with low intensity, enhances the differentiation of stem cells into osteoblasts. This technique is commonly used in clinical settings to treat bone fractures and defects. Ultrasound-activated p38 and AKT pathways are favorable for stem cell differentiation [165]. Even in microgravity environments, the osteogenic effects of ultrasound are still practical, indicating its potential in treating osteoporosis. Ultrasound effectively increased ALP production, upregulated OPN-OCN, and stimulated the Osx-Rankl/Runx2 pathway [166].

Continuous exposure to specific ultrasound frequencies promotes osteogenesis in human adipose-derived MSCs. Ultrasound stimulation can induce human mesenchymal stem cells to produce the soluble receptor activator of nuclear factor-kappa B ligand, enhancing osteoblastogenesis over time [167]. Using ultrasound stimulation is highly effective in manipulating stem cells in clinical and biophysical contexts. The implications of ultrasound frequencies have considerable practical applications in the present and future (Fig. 6d).

Light wavelengths substantially impact stem cell behavior and regenerative capacity, especially in bone regeneration. Phototherapy induces the ERK phosphorylation pathway in stem cells (Fig. 6e), increasing cell proliferation [168]. Subsequently, light stimulation can be a valuable tool for enhancing bone regeneration, although the appropriate wavelength must be selected.

In many cases, a combination of different external stimulation was effective. Adding electrical stimulation to the topography and controlled cell culture environments may improve bone and muscle regeneration. Therefore, one method can control direction and cell morphology through delicate patterning, while additional techniques can achieve directed cell differentiation and migration.

5.4. Clinical trials of electrical stimulation

Various clinical trials have investigated external stimulation for therapeutic interventions, with each modality offering unique insights into its respective applications (Table 4). Importantly, one study explored the effects of electrical stimulation on hemodynamic physiology. The study used a stimulation frequency of 1 Hz, a pulse duration of 1 ms, and a pulse time of 5 min. These settings increased venous velocity in the ipsilateral femoral vein and heightened fluximetry signals in the skin of both feet. Researchers found that the discomfort associated with stimulation could be finely controlled by adjusting the applied current [169]. Another trial demonstrated the potential of ultrasound in addressing muscle.

spasms. The low-intensity continuous ultrasound used in the study showed efficacy in mitigating myofascial pain [170]. In the magnetic fields, a study focused on conditions such as hereditary spastic

paraplegia and adrenomyeloneuropathy utilized 10 Hz repetitive transcranial magnetic stimulation. The study revealed enhanced signaling in the BDNF-TrkB complex, upregulation of NMDA receptors, increased muscle strength in both proximal and distal muscles, and reduced stiffness in proximal muscles [171]. Two notable studies explored electrical stimulation in the context of anterior cruciate ligament reconstruction and pulsed electromagnetic field therapy for musculoskeletal pain. The first study demonstrated that neuromuscular electrical stimulation reduced atrophy in skeletal muscle fibers [172], while the second showed that the combined treatment with osteopathic manipulative treatment and the electromagnetic field was more effective than either treatment alone (NCT04704375). A study on fibromyalgia employed the Re-Timer® device for light therapy. Remarkably, treatment with both bright and dim light yielded similar levels of improvement between the two groups (NCT03794908). Continuing the exploration of light therapy, a study investigated the use of Erchonia® GVL laser with green and violet wavelengths for musculoskeletal pain. The study highlighted the maximized therapeutic effects achieved through photochemistry, substantially reducing visual analog pain scores for neck and shoulder pain (NCT04895618). Yet another study focused on electrical stimulation for muscle atrophy and weakness. The authors observed a temporary decrease in oxyhemoglobin, swiftly followed by immediate recovery, indicating muscle activation during electrical stimulation (NCT05198466). Collectively, these trials contribute valuable knowledge to the evolving landscape of external stimulation therapies, showcasing their diverse applications across a spectrum of medical conditions.

Table 4

Summary of recent clinical trials of external stimulations.

External stimulation	Condition	Treatment Conditions	Description	Completed	Identifier
Electrical stimulation	Hemodynamic Physiology	Stimulation frequency: 1 Hz Pulse duration: 1 ms Pulse time: 5 min	- Electrical stimulation increases venous velocity in the ipsilateral femoral vein and increases fluximetry signals in the skin of both feet.	August 2015	NCT02532556
	Anterior Cruciate Ligament Reconstruction	Stimulation frequency: 50 Hz Pulse duration: 400 μs Pulse time: 50 min	- Neuromuscular electrical stimulation reduced the atrophy of skeletal muscle fibers of MHC II fibers and increased the maximum shortening velocity of MHC I fibers.	September 2019	NCT02945553
	Muscle Atrophy, Muscle Weakness	Stimulation frequency: 20–121 Hz Pulse duration: 400–1400 μs Pulse time: 1h	- The electrical stimulation caused a decrease in oxyhemoglobin, but the oxygen level immediately recovered after the stimulation, indicating that the muscle was activated.	August 2022	NCT05198466
Ultrasound	Muscle Spasm	Low-intensity continuous ultrasound (0.132W/cm^2, 3 MHz, 100 % duty cycle for 4 h, 18,720 J per treatment)	- Low-intensity continuous ultrasound to demonstrate decreased myofascial pain.	September 2015	NCT02135094
Light	Fibromyalgia	Re-Timer® Bright light: ~500 nm, 230 μW/m^2, 500 lux Dim: 3 μW/m^2, 7 lux	- Treatment with bright and dim light resulted in significantly similar levels of improvement between the two groups, though not dramatically different.	July 2021	NCT03794908
	Musculoskeletal Pain	Erchonia® GVL laser Wavelength: 520 nm (green), 405 nm (violet)	- Photochemistry, which involves light absorption by tissues, maximizes the therapeutic effects of green and violet wavelengths. - Effectiveness significantly reduced visual analog pain scores for neck and shoulder pain from 71.79 to 34.02.	May 2022	NCT04895618
Magnetic field	Hereditary Spastic Paraplegia, Adrenomyeloneuropathy	10 Hz repetitive transcranial magnetic stimulation	- Stimulation of the magnetic field at the cellular level enhances BDNF-TrkB complex signaling and upregulates NMDA receptors, providing remodeling of central motor pathways. - Muscle strength increased in proximal and distal muscles after stimulation, and stiffness decreased in proximal muscles.	January 2019	NCT03627416
	Musculoskeletal Pain	pulsed electromagnetic field	- Combined treatment with osteopathic manipulative treatment and electromagnetics for treating lower back pain was more effective than either treatment alone, with a mean reduction of 26.2 ± 28.8 after 3 weeks.	September 2019	NCT04704375

6. Research on bioactive materials for combined bone-muscle regeneration

Recognizing the critical relationship between bone and muscle, their regeneration must be considered as part of a continuum rather than isolated events. A prime example of this integration is observed at the enthesis, where muscle tendons connect to bone [173]. This is one of the primary functions of the musculoskeletal system, with bones, tendons, and muscle tissue working harmoniously to provide body movement [174]. Here, bioactive materials designed to mimic the gradient from soft to hard tissue provide necessary cues for simultaneous tendon and bone healing, thereby preserving the functional unity of the musculoskeletal system. Mimicking the biological and biomechanical properties of native tissue is not just a scientific challenge but also a crucial step toward effective regeneration. For biological movement, musculoskeletal cells should be effectively recruited in an environment that mimics their mechanical strength and structural features.

Recent breakthroughs in bioactive materials have paved the way for solutions that can simultaneously foster the regeneration of both bone and muscle [175]. Composite fibrous scaffolds, which are known for their biocompatibility and ability to mimic the properties of native tissue, promoted the differentiation of stem cells into both osteoblasts and myoblasts [176]. Specific biochemical cues such as BMPs and mechanical signaling influence this dual differentiation potential. While structurally distinct, the tissue matrix of the musculoskeletal system shares common elements, including collagens. Bones feature regular deposits of collagen type I, while muscles are enveloped by a collagen matrix (collagen types I and III) surrounding the muscle fibers. Given the ability of collagen to enhance biocompatibility and cellular response, it is a promising material for promoting musculoskeletal tissue regeneration. By leveraging the natural components of the ECM to mimic the bone-muscle interface, these materials bolster the structural integrity of regenerated tissues and facilitate functional recovery by aligning new tissue growth with existing biomechanical and biochemical cues.
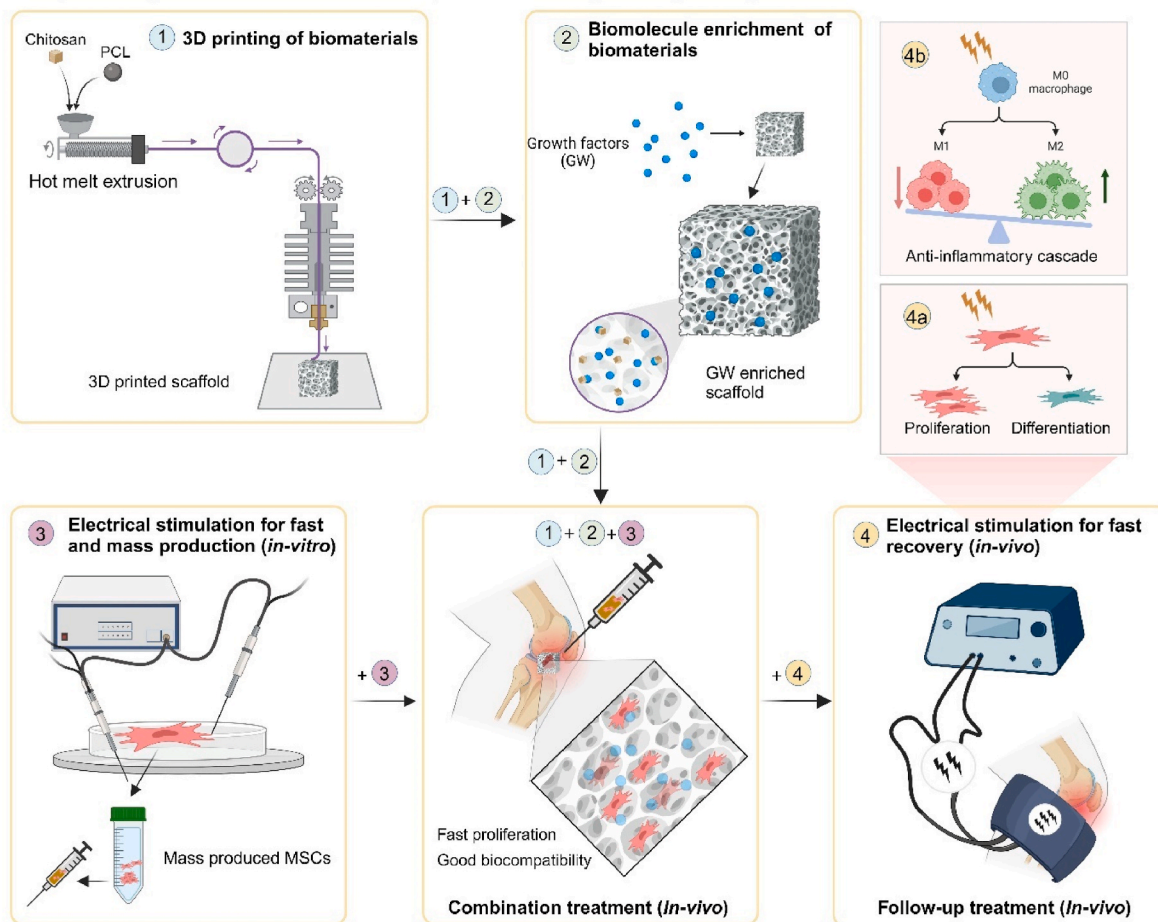
7. Combination of multiple strategies

Integrating multiple strategies for tissue regeneration is a comprehensive approach that effectively addresses the complex nature of musculoskeletal disorders. The multifaceted approach combines cell therapy, biomaterials, and biomolecules or external stimuli to exploit their synergistic effects, thereby significantly enhancing the overall efficacy of regenerative treatments (Fig. 7). A novel approach in tissue engineering involves combining multiple strategies, which can potentially improve the speed and effectiveness of tissue repair and regeneration, as shown in Fig. 8. This figure illustrates the integration of advanced biomaterials, growth factors, electrical stimulation, and 3D printing to create a synergistic environment conducive to tissue regeneration.
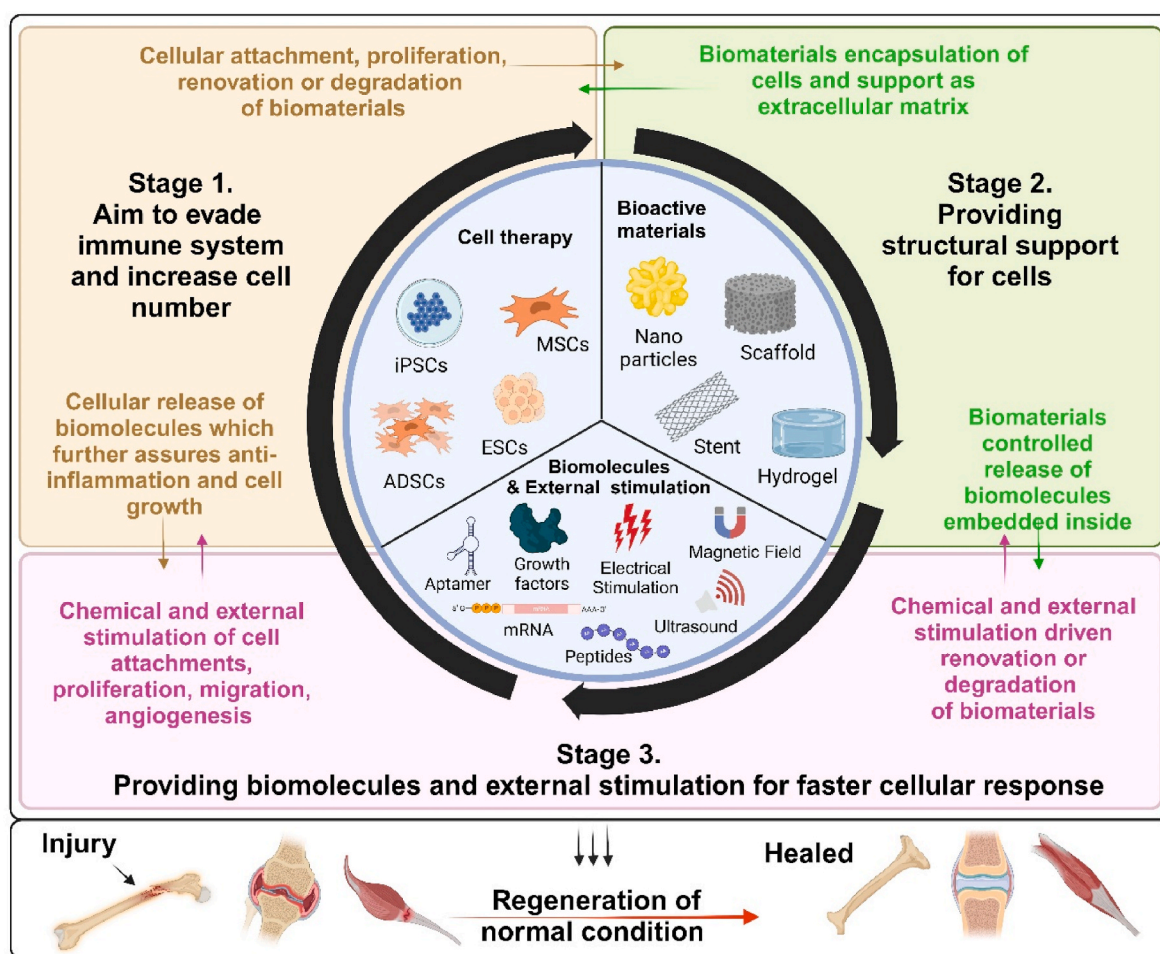
Fig. 7. Scheme for treating bone and muscle musculoskeletal disease using regenerative medicine and tissue engineering. Musculoskeletal disorders can be treated by combining techniques that involve evading and harnessing the immune system, providing structural support, and regeneration. These three targets are achieved using interconnected components such as cell therapy, biomaterials, and biomolecules. For example, MSCs can manipulate the immune system to prevent it from attacking healthy tissue, while scaffolds or other biomaterials provide structural support. Finally, regeneration can be achieved using specific medications or biological agents, such as growth factors and mRNA, that promote bone and muscle tissue regeneration.

Fig. 8. Illustration of multiple strategies combined to achieve fast and improved tissue engineering and regeneration. First, 3D printing creates scaffolds from chitosan and PCL via hot melt extrusion. These scaffolds are then enriched with growth factors to promote cell proliferation and differentiation. In vitro electrical stimulation is applied to MSCs to enhance their proliferation, aiding mass production. For in vivo applications, electrical stimulation accelerates tissue recovery by modulating cellular activities and promoting anti-inflammatory responses. The integrated approach facilitates the tailoring of treatments to meet the specific requirements of individual patients and the characteristics of their musculoskeletal disorders. This method signifies a sophisticated and comprehensive grasp of tissue engineering and regenerative medicine, offering the potential for more effective and long-lasting solutions for those affected by various musculoskeletal conditions.

Three-dimensional (3D) printing, specifically hot melt extrusion, fabricates scaffolds from biomaterials such as chitosan and poly-caprolactone [177]. This technique allows for precise control over the scaffold architecture, including porosity and mechanical properties, which are essential for mimicking the ECM and providing structural support for tissue growth. To enhance their bioactivity, scaffolds can be enriched with biomolecules, including growth factors. Growth factors like TGF-β can be embedded within the scaffold matrix to create a conducive cell proliferation and differentiation environment [178]. The enriched scaffold releases these factors gradually, maintaining a sustained biological effect that promotes tissue regeneration [179]. The combination creates an ideal environment for the proliferation and differentiation of MSCs, providing optimal conditions for their growth and development (Fig. 8$_{1+2}$).

Electrical stimulation is a promising technique and can be applied during in vitro culture to enhance the proliferation and differentiation of MSCs [180]. This pre-treatment approach helps in the mass production of MSCs with improved regenerative potential (Fig. 8$_3$). The electrical cues can mimic the physiological conditions that cells experience in vivo, thus enhancing their functionality [181]. Electrical stimulation can also be applied in vivo as a follow-up treatment to accelerate tissue recovery (Fig. 8$_4$) [182]. It can modulate cellular activities such as proliferation and differentiation (Fig. 8$_{4a}$) and influence macrophage polarization towards a pro-regenerative phenotype (M2) [183]

(Fig. 8$_{4b}$), which is crucial for resolving inflammation and promoting tissue repair.

The comprehensive treatment strategy shown in Fig. 8 (Fig. 8$_{1+2+3+4}$) is a testament to the power of collaboration in scientific research. By integrating 3D-printed scaffolds, biomolecule enrichment, and electrical stimulation both in vitro and in vivo, we can enhance cell proliferation, differentiation, and tissue regeneration, leading to faster recovery and improved clinical outcomes. This collaborative effort, using growth factor-enriched scaffolds combined with electrical stimulation, ensures good biocompatibility and creates an optimal environment for tissue repair.

Combining advanced biomaterials, growth factors, and external stimulation provides a multifaceted approach to tissue engineering [184]. The synergistic strategy leverages the strengths of each component, resulting in enhanced tissue regeneration and improved patient outcomes. Importantly, ongoing research and clinical studies can further refine these techniques and validate their efficacy in therapeutic applications. The integrated approach allows for the customization of treatments based on the specific needs of the patient and the nature of the musculoskeletal disorder. It represents a more advanced and nuanced understanding of tissue engineering and regenerative medicine, promising more effective and enduring solutions for patients suffering from various musculoskeletal conditions.

8. Prospects

The increasing demand for musculoskeletal treatments underscores the need for advanced therapeutic approaches. While natural healing processes are limited in reversing substantial musculoskeletal damage, regenerative medicine and tissue engineering present promising avenues for addressing severe injuries. Current research predominantly employs MSCs and ADSCs as primary cell sources [185]. MSCs, extensively studied in clinical trials, face limitations due to their scarcity and slow proliferation rates compared to other cell types. These factors can significantly impact the scalability and cost-effectiveness of MSC-based therapies; however, these limitations can potentially be overcome via advancements in faster in-vitro proliferation technologies. Conversely, ADSCs, which can be derived in large quantities from adipose tissue, are promising; however, they exhibit inconsistencies in musculoskeletal regeneration compared to MSCs and pose some safety concerns. The lack of standardized ADSC isolation and preparation protocols can contribute to these inconsistencies. Research is needed to improve ADSC effectiveness and address these concerns [186]. Further investigation is crucial to determine the fate of injected stem cells and whether they persist in the body to support regeneration or actively contribute to the regenerative process. Future research should focus on optimizing these stimuli to determine their impact on cell morphology and differentiation. Additionally, therapies that facilitate the fusion of regenerated tissue with existing tissues are essential for promoting faster musculoskeletal regeneration. However, to truly benefit patients with musculoskeletal disorders, comprehensive studies are required. Collective efforts toward refining these therapies can fundamentally change the lives of patients suffering from musculoskeletal disorders. Lastly, researchers must embrace new methods and continue to explore the strategies discussed in this review. To further advance tissue engineering and regenerative medicine, it's necessary to consider laser-induced modification of biomaterials and treatments [187], CAR T cell-mediated treatments [188], CRISPR-powered techniques [189], 3D printing for advanced biomaterials, and multi-omics analysis [190]. These innovative approaches can potentially overcome current limitations and significantly enhance the effectiveness of regenerative therapies.

9. Review methodology

This study highlights the crucial role of tissue engineering and regenerative medicine in treating various disorders. To conduct this review, we concentrated exclusively on three main strategies: cell therapy, biomaterials, and the additional regenerative power provided by growth factors and external stimulation. We extensively researched relevant scholarly articles published between January 2014 and December 2023. We only considered articles written in English and published in peer-reviewed journals.

Additionally, we searched clinical.gov to find completed clinical trials. Our search for articles and trials was conducted from January 1, 2014, to December 2023.

10. Conclusion

This literature review focuses on the challenges and opportunities of combining tissue engineering and regenerative medicine strategies. With this review, we aimed to equip new researchers with a deep understanding of the fundamental principles in these fields. The increasing use of combined strategies in tissue engineering and regenerative medicine presents a promising avenue for repairing and replacing damaged tissues and organs. The present review meticulously examines the current state-of-the-art techniques, their applications, and the challenges that must be overcome for further progress in the field.

CRediT authorship contribution statement

Soyeon Park: Writing – original draft, Methodology, Investigation, Formal analysis. **Khandoker Asiqur Rahaman:** Writing – review & editing, Resources, Formal analysis, Data curation. **Yu-Chan Kim:** Supervision, Resources, Conceptualization. **Hojeong Jeon:** Validation, Supervision, Conceptualization. **Hyung-Seop Han:** Writing – review & editing, Writing – original draft, Supervision, Conceptualization.

Declaration of competing interest

Hyung-Seop Han is an editorial board member for Bioactive Materials and was not involved in the editorial review or the decision to publish this article. All authors declare that there are no competing interests.

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