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Functionalized mesenchymal stem cells for enhanced bone regeneration: advances and challenges

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Abstract

Bone fracture continues to pose a significant clinical challenge in regenerative medicine due to limited repair capacity and inadequate therapeutic options. Among the various therapeutic strategies, mesenchymal stem cells have shown strong potential due to their ability to promote bone formation, modulate inflammation, and migrate to injury sites. However, clinical outcomes have been limited by issues like low survival rates, poor integration, and non-specific distribution after transplantation. Functionalized mesenchymal cells enhanced through genetic, chemical, or material-based modifications have emerged as an advanced strategy to overcome these limitations and significantly improve bone regeneration. This review explores recent developments in the functionalization of stem cells to increase their bone-forming potential. It covers techniques of gene modification, preconditioning, nanoparticle integration, and scaffold-based delivery. The role of these engineered cells is in activating key pathways involved in bone repair, including bone morphogenetic proteins and Wnt signaling. Furthermore, the study highlights current delivery platforms, including injectable gels, printed scaffolds, and bioactive coatings that support targeted, sustained cell activity. Despite encouraging preclinical outcomes, unresolved challenges in manufacturing, immune compatibility, and regulatory pathways persist, prompting the exploration of emerging solutions like precision-engineered implants and artificial intelligence-driven design to guide the future of advanced bone regeneration therapies.

Keywords Mesenchymal stem cells, Osteogenesis, Bone fracture healing

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Introduction

Bone fractures are among the most common injuries encountered in clinical practice, yet their treatment becomes particularly challenging in cases involving critical-sized defects, delayed union, non-union, or patients with systemic conditions such as osteoporosis, diabetes, or cancer [1]. Conventional therapeutic strategies including autologous and allogeneic bone grafts, present significant limitations like donor site morbidity, limited graft availability, immune rejection, and variable rates of graft integration [2, 3]. Moreover, synthetic biomaterials, while useful as temporary scaffolds, often lack osteoinductive potential and fail to fully recapitulate the complex cellular signaling required for robust bone regeneration [4]. In recent years, regenerative medicine has shifted its focus toward biologically active approaches that support and stimulate the body's intrinsic healing mechanisms [5]. Among these, stem cell–based therapies have emerged as a transformative strategy to promote bone repair. Mesenchymal stem cells (MSCs), in particular, have gained widespread attention due to their multipotent nature, ease of isolation from various tissues (bone marrow, adipose tissue, umbilical cord), and their ability to differentiate into osteoblasts, chondrocytes, and adipocytes [6]. More importantly, MSCs exert strong paracrine effects by secreting cytokines, growth factors, and exosomes that promote angiogenesis, immunomodulation, and extracellular matrix remodeling key components in the bone regeneration cascade [7].

The biological rationale for using MSCs in bone healing is rooted in their capacity to participate in both direct and indirect mechanisms of osteogenesis [8]. Directly, MSCs contribute by differentiating into bone-forming osteoblasts under appropriate biochemical and mechanical stimuli. Indirectly, they secrete trophic factors such as bone morphogenetic proteins (BMPs), vascular endothelial growth factor (VEGF), transforming growth factor-beta (TGF-β), and insulin-like growth factors (IGFs), which modulate local cell behavior and promote tissue repair [9]. Furthermore, MSCs display immunosuppressive properties, which make them particularly valuable in allogeneic applications, reducing the risk of host immune rejection [10]. Despite their promise, the clinical translation of MSC-based therapies faces significant challenges. Unmodified or "naïve" MSCs often exhibit poor survival and low engraftment rates following transplantation, especially in ischemic or inflammatory environments such as those present in non-union fractures [11]. Additionally, their osteogenic potential may be inconsistent due to donor variability, passage number, and culture conditions. These limitations have prompted the exploration of MSC modification a broad term encompassing various strategies aimed at enhancing the therapeutic efficacy of MSCs [12]. The field is also evolving to integrate these modified MSCs within bioactive delivery platforms such as injectable hydrogels, electrospun scaffolds, and 3D-printed constructs that provide mechanical support while promoting cell viability and spatial control [13, 14]. Moreover, advances in nanotechnology and biofabrication have enabled the development of "smart" scaffolds that can respond to environmental cues (pH, temperature, enzymatic activity) to release bioactive molecules in a controlled fashion, further improving the regenerative microenvironment [15]. Understanding how preconditioned MSCs interact with their microenvironment, deliver osteogenic cues, and integrate with host tissues is essential for developing next-generation bone regeneration therapies [16].

This review aims to provide a comprehensive synthesis of recent developments in the optimization of mesenchymal stem cells for bone fracture healing. It explores the biological underpinnings of MSC-mediated osteogenesis, analyzes emerging strategies for enhancing their regenerative performance, and evaluates the efficacy of various delivery platforms. In doing so, the review highlights major challenges including immune safety, large-scale production, and regulatory considerations while also discussing future directions such as the integration of artificial intelligence, precision-engineered implants, and patient-specific therapies that promise to redefine the clinical landscape of bone regeneration.

Biological and therapeutic profile of mesenchymal stem cells

MSCs are multipotent stromal cells derived from various adult and perinatal tissues, including bone marrow, adipose tissue, and umbilical cord [17] (Fig. 1). While bone marrow-derived MSCs (BM-MSCs) remain the most studied due to their high osteogenic potential, adipose-derived MSCs (AD-MSCs) and umbilical cord-derived MSCs (UC-MSCs) offer significant clinical advantages. AD-MSCs are easier to harvest via minimally invasive procedures (e.g., liposuction), yield substantially higher cell numbers (over 5000 cells per gram of adipose tissue vs. 100–1000 MSCs per mL of bone marrow), and exhibit greater proliferative capacity in vitro. UC-MSCs can be collected painlessly from discarded umbilical cords, are ethically unencumbered, and demonstrate the highest proliferation rates and clonogenic potential among MSC sources, with reduced expression of senescence markers (p53, p21, p16). These unique attributes make AD-MSCs and UC-MSCs highly attractive for scalable regenerative therapies, particularly when large cell doses are required [18–21].

Recent studies recognize dental pulp stem cells (DPSCs) as a promising MSC source for bone regeneration due to their multipotency, abundant availability, and minimally invasive harvestability. DPSCs exhibit robust

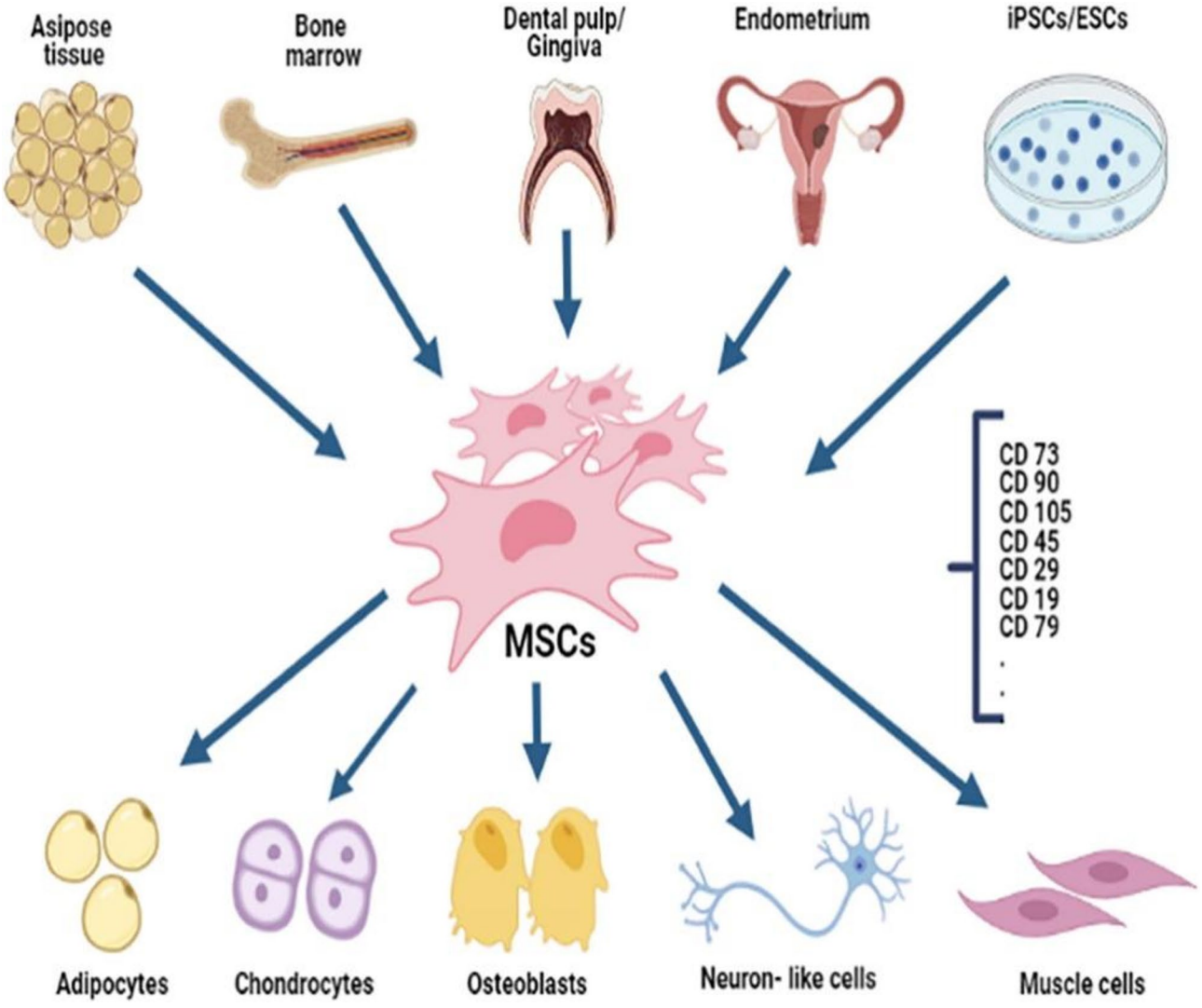

Fig. 1 MSCs can be isolated from different sources, neonatal, fetal, and adult tissues. These cells possess the potential, under specific conditions, to differentiate into a wide range of cell types, including osteoblasts, chondrocytes, and adipocytes, making them a promising candidate for regenerative therapies across multiple tissue types.
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osteogenic differentiation in vitro, expressing markers such as ALP, type I collagen, BMP-2/4, osteonectin, osteopontin, and osteocalcin and can form mineralized nodules under osteoinductive culture conditions [22]. In vivo meta-analyses have demonstrated that DPSC-based interventions significantly enhance new bone area and volume compared to controls [23]. A 2024 review further emphasizes DPSCs' clinical appeal, citing their easy accessibility, low immunogenicity, and potential for both cell-based and cell-free bone regenerative strategies [24]. Moreover, scaffold-based in vivo studies reveal that combining DPSCs with biomaterials can achieve nearly complete structural restoration of critical bone defects, resulting in well-vascularized lamellar bone formation, increased TGF-β1 levels, and a conducive bone microenvironment [25]. MSCs are typically identified by the expression of surface markers including CD73, CD90, and CD105, along with the absence of hematopoietic markers like CD34, CD45, and HLA-DR [26, 27]. However, variations in these markers have been observed depending on the tissue source and culture conditions, indicating phenotypic heterogeneity that may influence therapeutic performance [28, 29]. MSCs possess robust differentiation capacity, particularly into osteogenic lineages under osteoinductive conditions, making them highly attractive for bone tissue engineering [30, 31]. This osteogenic differentiation is tightly regulated by transcription factors such as RUNX2 and signaling pathways including Wnt/β-catenin and BMP [32–34]. In addition to their regenerative capacity, MSCs play a pivotal role in

immunomodulation, exerting anti-inflammatory effects by inhibiting T-cell proliferation, inducing regulatory T-cells, and secreting immunosuppressive molecules like prostaglandin E2, TGF-β, and interleukin-10 [35, 36]. These properties enhance their therapeutic utility in bone healing, particularly in inflammatory or immune-compromised conditions.

MSCs exhibit notable variability in proliferative potential depending on their tissue of origin, which significantly affects their suitability for clinical-scale applications. For example, umbilical cord-derived MSCs (UC-MSCs) display markedly higher population doubling limits and faster expansion rates compared to BM-MSCs and AD-MSCs [38]. Conversely, in a comparative study of canine MSCs, adipose-derived MSCs (AD-MSCs) demonstrated the shortest population doubling time, indicating superior in vitro growth rates [39]. Donor characteristics such as age profoundly influence MSC proliferation. Placenta-derived MSCs from donors aged 22–35 showed enhanced growth rates and colony-forming efficiency relative to cells from younger or older donors [40]. Moreover, aging reduces MSC proliferative potential across tissues; younger donors retain superior self-renewal capacity [20]. Culture conditions and source-dependent heterogeneity also modulate proliferative behavior. Factors such as culture density, oxygen tension, and cell isolation methods significantly affect replicative potential and senescence onset [41].

Clinically, MSCs have been investigated for various orthopaedic applications, including non-union fractures, critical-sized bone defects, osteonecrosis of the femoral head, spinal fusion, and bone cysts (Fig. 2) [42, 43]. Early-phase clinical trials have reported encouraging results, with MSC-based therapies promoting bone regeneration, reducing pain, and minimizing the need for autologous

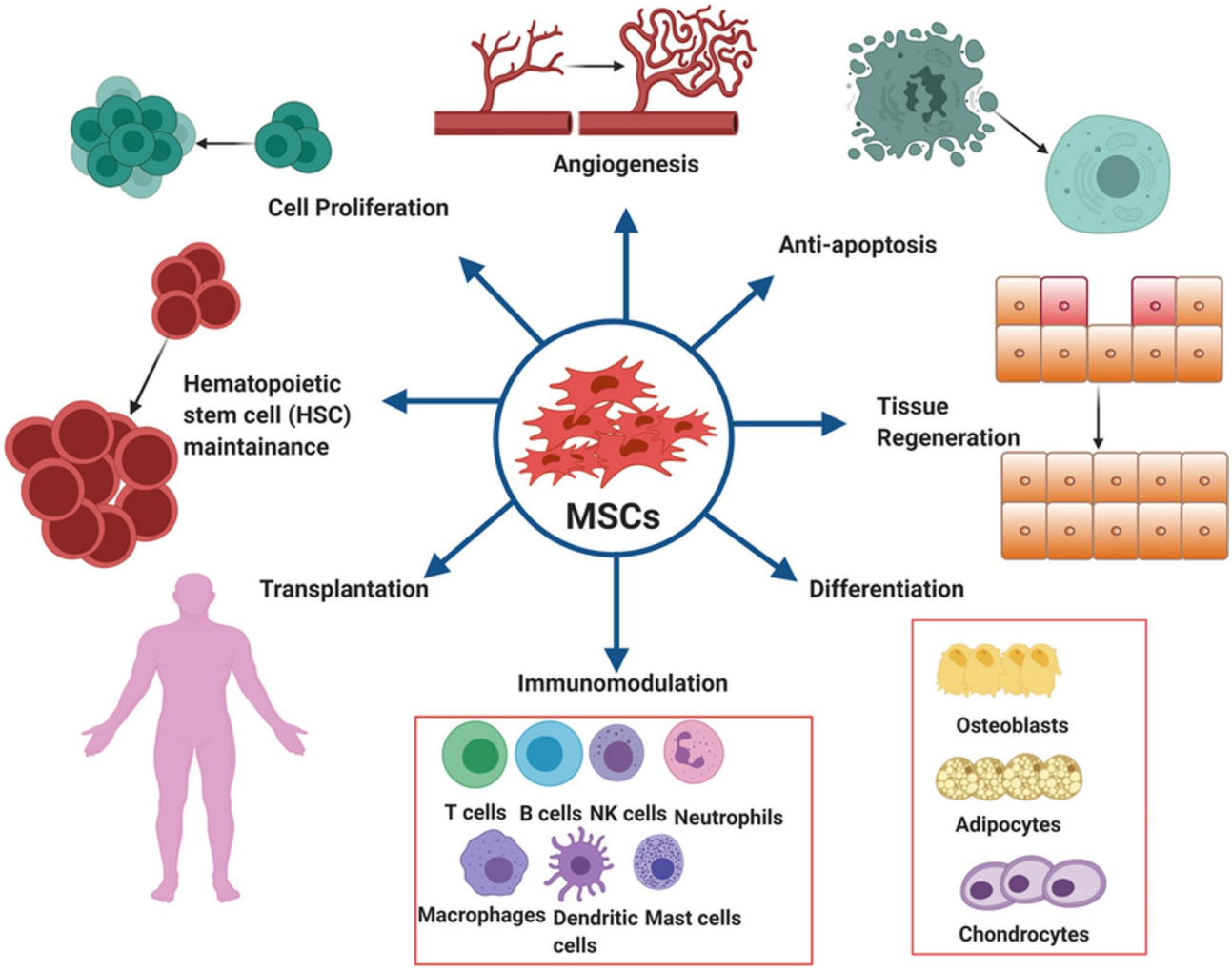

Fig. 2 Schematic representation of the functions of mesenchymal stem cells (MSCs) in biological systems.
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grafts [44, 45]. However, despite these promising outcomes, several challenges persist, particularly regarding donor-to-donor variability, large-scale manufacturing, immune compatibility, and regulatory approval [46]. Moreover, the translation of MSC therapies from bench to bedside is limited by the lack of standardized protocols and variability in delivery methods [47]. Nonetheless, ongoing developments in biomaterial engineering, scaffold design, and cellular functionalization continue to improve the osteogenic performance and clinical applicability of MSCs in bone tissue repair [48].

Osteogenesis and bone healing cascade

Following a fracture, the repair cascade begins with an inflammatory phase characterized by hematoma formation and infiltration of immune cells, which release cytokines and chemokines that recruit MSCs to the injury site [50]. Immune cells release a range of cytokines and chemokines, including interleukins (IL-1, IL-6, IL-11, IL-18), tumor necrosis factor-alpha (TNF-α), monocyte chemoattractant protein-1 (MCP-1), and platelet-derived growth factor (PDGF), among others, to initiate the inflammatory phase of fracture healing (Fig. 3). The subsequent repair phase involves proliferation and osteogenic differentiation of MSCs, as well as angiogenesis and extracellular matrix deposition. MSCs play a central role by differentiating into osteoblasts, which are responsible for new bone formation [51]. This differentiation is governed by complex signaling networks, including the activation of RUNX2, a master transcription factor required for commitment to the osteogenic lineage [52, 53]. Other critical pathways include Wnt/β-catenin, which promotes osteoblast maturation and mineralization; bone morphogenetic protein-2 (BMP-2), which enhances early osteogenic induction; and the Notch signaling pathway, which contributes to osteogenic regulation through interaction with the bone microenvironment [54, 55]. In addition to their osteogenic role, MSCs support neovascularization by secreting pro-angiogenic factors such as VEGF, thereby ensuring adequate nutrient and oxygen supply essential for bone tissue regeneration [56]. They also contribute to matrix remodelling by producing enzymes like MMP-2 and MMP-9, facilitating the transition from a soft callus to a mineralized bone structure [57]. Through their combined effects on bone formation, angiogenesis, and remodelling, MSCs function as orchestrators of the bone healing cascade, making them key targets

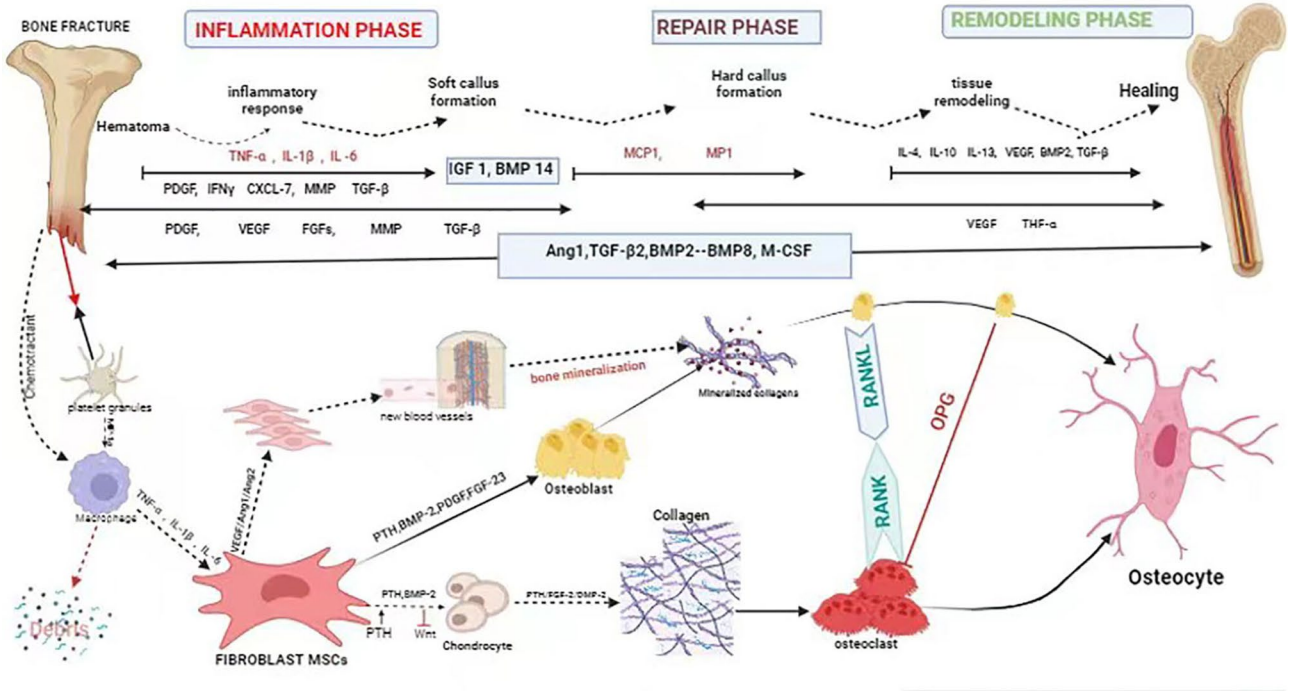

Fig. 3 Schematic representation of the sequential phases of fracture healing—namely inflammation, repair, and remodeling—highlighting the dynamic involvement of mesenchymal stem cells (MSCs) and key growth factors. The healing cascade includes hematoma formation, immune cell recruitment, soft and hard callus formation, and eventual bone remodeling. PDGF facilitates MSC recruitment and proliferation; VEGF drives angiogenesis; BMP-2 promotes osteoblast and chondrocyte differentiation; FGF-2 supports matrix synthesis and mitogenic activity; IGF and TGF-β regulate osteoblast and osteoclast function during bone regeneration.

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for enhancing fracture repair and addressing non-union defects [58].

Following the early phases of inflammation and osteogenesis, the consolidation phase (days 21–42) transitions the soft callus into a mechanically stable, mineralized hard callus, driven by robust angiogenic signaling notably VEGF-A and angiopoietin-2 which promote vascular invasion and matrix mineralization. Thereafter, during the remodeling phase (from ~ day 42 to and beyond day 64), woven bone is gradually replaced by organized lamellar bone through tightly regulated resorption and formation dynamics mediated by the RANKL–OPG axis; specifically, VEGF enhances osteoblast proliferation and upregulates RANKL while downregulating OPG, thereby indirectly promoting osteoclastogenesis and coordinated bone turnover. Meanwhile, paracrine/autocrine VEGF signaling via VEGFR1/VEGFR2 influences osteoblast and endothelial cross-talk, further supporting remodeling and angiogenesis of the callus [59–62].

Strategies for functionalizing MSCs to enhance osteogenesis

To enhance the therapeutic efficiency of MSCs in bone regeneration, a range of functionalization strategies have been designed to modulate their osteogenic potential through genetic, epigenetic, biophysical, and nanoengineering approaches [64].

Genetic engineering has emerged as a cornerstone strategy, wherein MSCs are transfected with osteoinductive genes such as *BMP-2*, *RUNX2*, and *VEGF* using viral or non-viral vectors (Fig. 4) [65]. These genetic interventions not only amplify the cells' intrinsic differentiation capacity but also enhance local angiogenesis, which is critical for mineralized tissue formation. Viral delivery methods, such as lentiviral and adenoviral vectors, have demonstrated high transfection efficiency; however, they also raise concerns regarding insertional mutagenesis and long-term safety, which remains a key regulatory challenge in clinical translation [66–68].

Epigenetic modulation offers a non-integrative strategy for altering MSC behavior. MicroRNAs such as *miR-26a*, *miR-29b*, and *miR-21* have been identified as potent regulators of osteogenesis through their ability to suppress inhibitory signaling pathways (GSK-3β, Smad7), thereby promoting the expression of osteogenic genes [69, 70]. Synthetic miRNA mimics or inhibitors, delivered via lipid nanoparticles or exosomes, have shown efficacy in preclinical fracture models, enhancing matrix mineralization and osteoblast marker expression [71, 72]. Recent studies underscore the therapeutic potential of mesenchymal stem cell-derived small extracellular vesicles (MSCs-sEVs) as delivery vehicles for microRNAs (miRNAs) in bone regeneration [73, 74]. By modulating gene expression, these miRNAs influence key cellular processes such as osteoblast proliferation, migration, and differentiation. For instance, sEVs enriched with miR-21-5p, miR-126-5p, miR-29a, and miR-27a-5p have demonstrated efficacy in enhancing osteogenesis, angiogenesis, and matrix mineralization through the regulation of targets like KLF3, VASH1, Atg4B, and IGFBP3 [73]. Several miRNAs within MSCs-sEVs have been shown to activate osteogenic

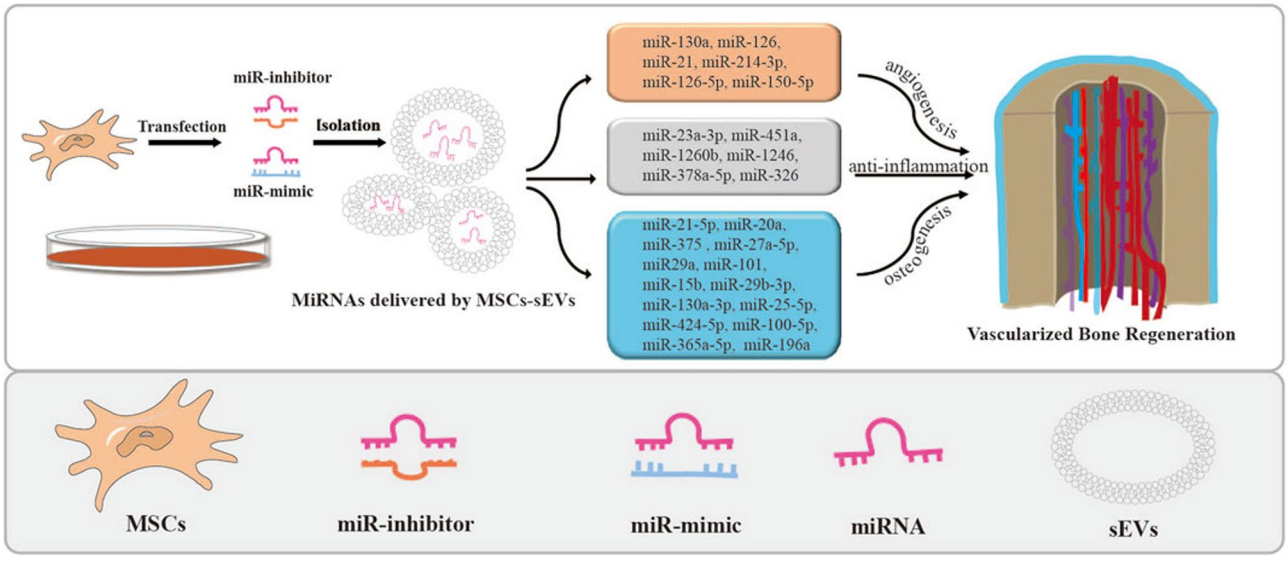

Fig. 4 Through transfection-based approaches, MSCs-derived small extracellular vesicles (sEVs) serve as carriers for miRNAs. By introducing miRNA mimics or inhibitors into stem cells, the expression levels of specific miRNAs within MSCs-sEVs can be upregulated or suppressed. These miRNA-enriched sEVs subsequently promote angiogenesis and facilitate bone regeneration.
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signaling pathways, including Wnt/β-catenin, PI3K/AKT, NF-κB, and RhoA/ROCK, while others inhibit osteogenesis by downregulating osteogenic markers (RUNX2, ALP, OCN) via pathways such as SMAD2/ERK and BMPR2/Smad1/5/9 [73]. Notably, long non-coding RNAs (lncRNAs) within sEVs further refine osteogenic responses by acting as competitive endogenous RNAs, sequestering miRNAs and thus promoting the expression of osteoinductive genes [75, 76].

Beyond osteogenesis, MSCs-sEVs play pivotal roles in suppressing osteoclast differentiation and promoting angiogenesis—critical for bone remodeling. miRNAs such as miR-21, miR-27a, and miR-6924-5p inhibit bone resorption, while others (miR-21/NOTCH1/DLL4 axis and miR-126/PI3K/AKT pathway) facilitate vascular development [73]. Additionally, sEVs contribute to immunomodulation by inducing M2 macrophage polarization and attenuating inflammation, thus creating a favorable microenvironment for bone repair [77]. These findings highlight the multifaceted role of MSCs-sEVs in orchestrating osteogenesis, angiogenesis, and immunomodulation via the targeted delivery of miRNAs and lncRNAs—underscoring their promise in regenerative medicine and bone tissue engineering [73].

Biophysical and biochemical priming strategies further enhance osteogenic readiness by exposing MSCs to tailored microenvironments [78]. Mechanical stimulation through cyclic strain or fluid shear stress has been shown to upregulate mechanosensitive pathways such as *YAP/TAZ* and integrin-FAK signaling, reinforcing osteogenic lineage commitment [79, 80]. Hypoxic preconditioning (1–5% O_2) mimics in vivo niche conditions and has been associated with enhanced secretion of angiogenic factors and improved cell survival [81, 82]. Biochemical cues, such as dexamethasone, β-glycerophosphate, and ascorbic acid, remain standard in vitro additives that direct osteogenic priming [83].

Nanoparticle-mediated functionalization introduces a versatile toolkit for MSC engineering, enabling both targeted delivery and real-time imaging [84]. Gold and silica nanoparticles modified with osteoinductive molecules (*BMP-2*, *RGD peptides*) allow for controlled release and localized activity at the defect site [85, 86]. Magnetic nanoparticles, particularly those coated with poly-L-lysine or gelatin, offer magnetically guided delivery, improving retention and spatial control (Fig. 5) [87, 88]. Recent studies also demonstrate that such nanoparticles can activate mechanotransduction pathways through magneto-mechanical stimulation, further augmenting osteogenesis [89]. However, the long-term safety of these nanomaterials requires more thorough discussion. Gold nanoparticles, despite their osteoinductive potential, exhibit dose-dependent cytotoxicity and can induce reactive oxygen species generation and membrane damage at high concentrations. Silica nanoparticle interactions with bone cells are highly dependent on size, surface chemistry, and protein corona formation, which can unpredictably alter cellular responses and toxicity profiles [90, 91]. Moreover, while magnetic nanoparticles such as SPIONs enable enhanced cell homing, tracking, and mechanotransduction, concerns remain regarding aggregation, potential iron-mediated oxidative stress, immune activation, and limited in vivo localization, particularly in deep tissues. Therefore, before predicting clinical viability, these long-term biocompatibility and safety considerations must be rigorously evaluated through dose–response studies, biodegradation assessments, and preclinical trials [91].

Beyond nanomaterials, calcium-based biomolecules like calcium chloride ($CaCl_2$) and amino-calcium (AC) are gaining attention for bone regeneration due to their osteoconductive properties. Amino-calcium has demonstrated significant bone-preserving effects in ovariectomized rats, enhancing bone volume and trabecular connectivity, while upregulating osteogenic markers such as IBSP and BGLAP in MC3T3-E1 cells. Similarly, $CaCl_2$, when integrated into bioceramic or polymeric scaffolds, improves compressive strength, mineralization, and cell adhesion. The long-term biodegradation, immunogenicity, and systemic effects of both AC and $CaCl_2$ remain insufficiently characterized. Thus, rigorous in vivo studies are essential to validate their biocompatibility and clinical safety [92–94].

Cell surface modification techniques have been developed to enhance homing efficiency and interaction with bone tissue [96]. Ligand-receptor strategies, such as conjugating MSC surfaces with RGD motifs, CXCR4, or integrin-targeting peptides, improve adherence to inflamed or injured endothelium and facilitate site-specific accumulation [97]. Biomimetic coatings using extracellular matrix proteins like fibronectin or osteopontin have been shown to increase integrin-mediated adhesion and downstream osteogenic signaling [98]. These multifaceted strategies underscore the progress toward precision MSC therapies. By integrating genetic, biochemical, physical, and surface-engineering tools, researchers are now able to fine-tune the therapeutic profile of MSCs to match the complex demands of bone healing, particularly in challenging clinical scenarios such as critical-sized defects or osteoporotic fractures [99].

MSC delivery platforms and bioengineering integration

Advanced delivery platforms and bioengineering strategies have been developed to optimize the efficacy of stem cell therapies for bone regeneration given in Table 1. 3D

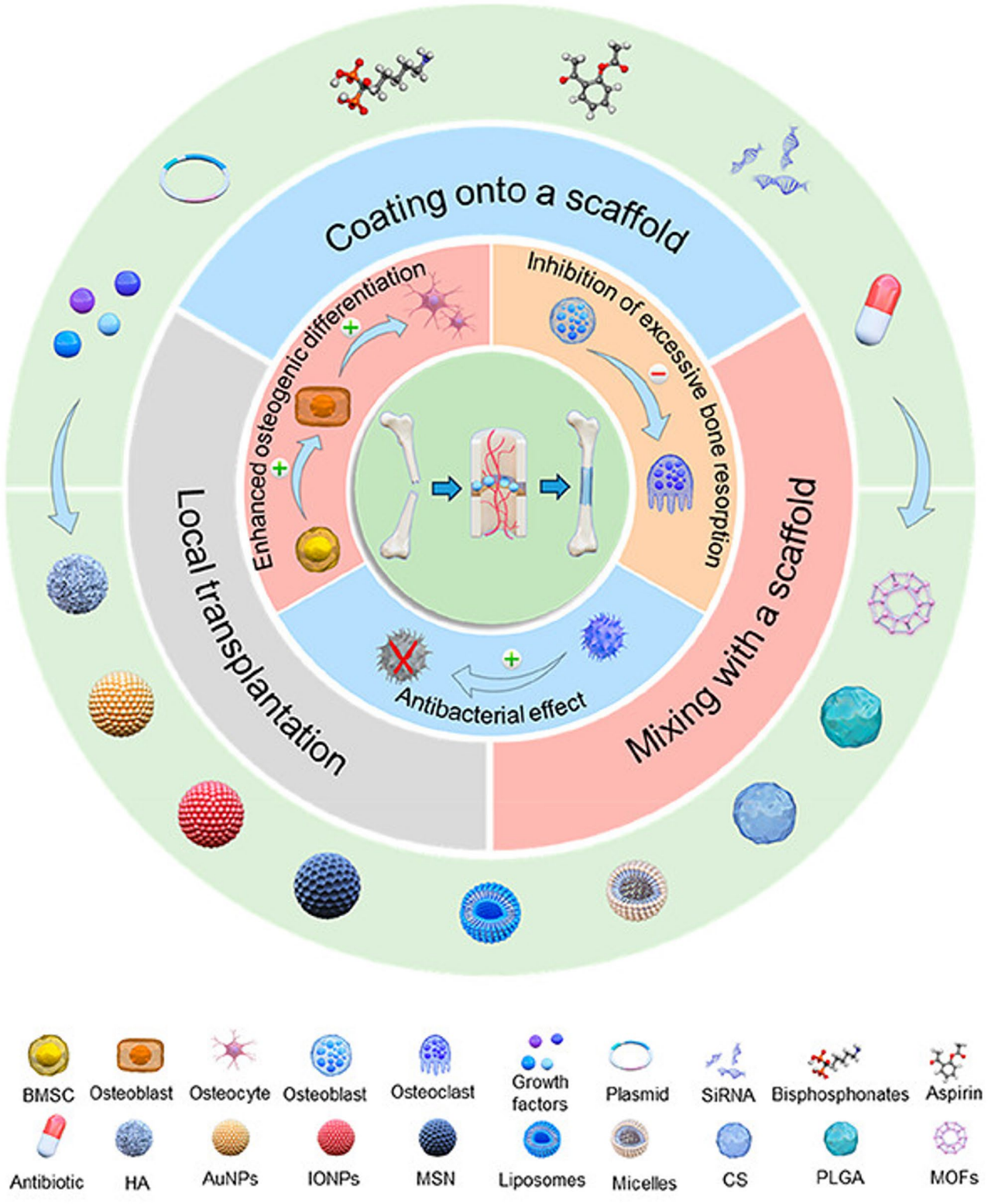

Fig. 5 Schematic representation of nanoparticle-mediated functionalization strategies. Nanoparticles serve as delivery platforms for bioactive molecules, enabling targeted modulation of cellular behavior and enhancing tissue regeneration through improved stability, cellular uptake, and controlled release of therapeutic agents.

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Table 1 Various MSC delivery platforms and bioengineering integration strategies for enhanced bone Regeneration, highlighting key features, advantages, and associated challenges in each approach

Delivery Platform	Key Features	Advantages	Challenges	References
3D Bioprinted Scaffolds	Customizable 3D-printed scaffolds for precise bone defect repair.	High precision, supports cell growth, osteogenesis.	High cost, technical complexity.	[106]
Hydrogel and Injectable Carriers	Hydrogels used as injectable carriers for MSC delivery.	Easy to inject, adaptable to various defect shapes, biocompatible.	Limited mechanical strength, burst release.	[107]
Microcarrier-Based MSC Expansion	Biodegradable microcarriers for MSC expansion in culture.	Efficient cell expansion, scalable for clinical applications.	Limited long-term stability, handling challenges.	[108]
Biofunctionalized Implant Surfaces	Implants functionalized with bioactive molecules to enhance MSC adhesion.	Enhanced cell attachment and differentiation, improved integration.	Difficulty in maintaining bioactivity in vivo.	[109]
Exosome-Loaded Functionalized MSCs	MSCs loaded with exosomes for enhanced therapeutic effects.	Enhanced regenerative potential, targeted therapy.	Difficulty in exosome isolation, scalability issues.	[110]
Electrospun Nanofiber Scaffolds	Nanofibers mimicking the extracellular matrix for MSC culture.	High surface area, customizable fiber alignment, promotes osteogenesis.	Low scalability, issues with fiber orientation.	[111]
Microsphere-Based Drug Delivery	Biodegradable microspheres for controlled release of MSCs or growth factors.	Sustained release, enhanced cell survival, and targeted delivery.	Burst release, limited release control.	[112]
Nanostructured Biomaterial Coatings	Coating of implants with nanomaterials to enhance MSC interactions.	Improved MSC adhesion and osteogenic differentiation.	Coating consistency, immune responses.	[113]
Photopolymerized Hydrogels	Hydrogels that solidify under UV light, encapsulating MSCs.	Precise control over scaffold formation, biocompatible.	UV sensitivity, limited to certain applications.	[114]
Nanofiber-Encapsulated Cell Therapy	MSCs encapsulated in nanofiber mats for enhanced interaction with matrix.	Promotes differentiation and cell migration.	Production difficulty, cell viability concerns.	[115]
Self-assembled Peptide Hydrogels	Hydrogels that self-assemble from peptides, supporting MSC encapsulation.	Biocompatible, promotes osteogenesis, easy to functionalize.	Limited mechanical strength, stability issues.	[116]
ECM Mimicking Scaffolds	Scaffolds designed to mimic natural bone extracellular matrix.	Better MSC differentiation and integration into host tissue.	Difficulty in mimicking natural ECM properties.	[117]
Microfluidic Platforms for MSC Culture	Microfluidic devices providing controlled culture environments for MSCs.	Precise nutrient and waste control, supports MSC differentiation.	Complex design, scalability issues.	[118]

bioprinted scaffolds offer precise control over structural features, enhancing cell attachment, proliferation, and differentiation [100]. These scaffolds are often combined with osteoinductive factors to further support bone formation [101]. Hydrogel-based and injectable carriers provide flexibility and enable minimally invasive delivery, offering protection to cells while facilitating sustained release of bioactive molecules [102]. Microcarrier-based systems assist in cell expansion during culture and promote efficient transplantation, improving cell survival [103]. Biofunctionalized implant surfaces guide cell migration and differentiation, promoting tissue integration and osseointegration [104]. Additionally, exosome-loaded cells are emerging as a novel approach, using the bioactive molecules in exosomes to enhance tissue regeneration and improve therapeutic outcomes [105].

Pharmacokinetic and pharmacodynamic perspectives in optimized MSC-based bone regeneration therapies

Understanding the pharmacokinetics (PK) and pharmacodynamics (PD) of functionalized MSC therapies is essential for optimizing their clinical translation in bone regeneration [119]. Following systemic or local administration, MSC biodistribution is influenced by various factors, including cell surface modifications, delivery route, and the presence of inflammatory or hypoxic microenvironments [119–121]. A major pharmacokinetic limitation involves first-pass pulmonary entrapment, where over 80% of infused cells are sequestered in the lungs, limiting bioavailability at osseous defect sites [121]. Functionalization strategies such as SDF-1/CXCR4 axis modulation, integrin ligand incorporation, and biomaterial-assisted cell delivery have shown promise in enhancing homing efficiency and tissue-specific retention [122]. MSCs primarily exert their therapeutic effects via a paracrine mechanism, secreting a repertoire of cytokines, growth factors, and extracellular vesicles that modulate osteogenesis, angiogenesis, and immune responses [123, 124]. These enhancements such as gene editing (BMP-2 overexpression), miRNA modulation, and nanoparticle conjugation have been employed to prolong and potentiate these effects in vivo [125]. However, the transient viability of transplanted MSCs and the temporally limited nature of their secretome necessitate integration with sustained-release delivery systems to maintain therapeutic efficacy [126]. The delivery route critically affects both pharmacokinetics and regenerative efficacy. Local delivery via intraosseous injection, hydrogel scaffolds, or composite carriers—offers superior outcomes in bone healing contexts by enabling direct cell deposition at the

defect site, enhancing cell retention, and promoting sustained paracrine signaling within the bone microenvironment. This is especially important given bone's relatively low vascularity compared to neural or spinal tissues, which limits systemic MSC migration and engraftment in orthopedic indications [127]. Systemic delivery, while non-invasive and suitable for diffuse skeletal conditions, often results in off-target cell accumulation (especially in the lungs and liver) and requires strategies to enhance homing capacity to bone tissue. Moreover, systemic routes may exhibit unpredictable pharmacodynamics in poorly perfused osseous lesions, underscoring the importance of site-specific delivery platforms [128, 129].

PK/PD profiling standardization for cell-based products remains a critical gap in current translational pipelines [130]. Quantitative imaging, biodistribution assays, and systems pharmacology models are being developed to better predict cell fate, optimal dosing, and therapeutic windows [131]. As regulatory agencies move toward stricter guidelines for advanced therapy medicinal products (ATMPs), incorporating robust PK/PD frameworks will be pivotal in ensuring safety, efficacy, and batch-to-batch consistency in modified MSC therapies for bone repair [132].

Preclinical and clinical evidence

Preclinical studies play a pivotal role in evaluating the efficacy and safety of MSC-based therapies for bone regeneration. Animal models have often been used to assess MSC transplantation in bone fractures, critical-sized defects, and osteoporotic bone healing [133]. Bone Defects (Fractures, Critical-sized defects).

In fracture and defect models, MSCs have been shown to enhance callus formation, bridge critical-sized gaps, accelerate mineralization, and improve biomechanical strength, as assessed through BMD, histology, μCT, and mechanical testing. Notably, a systematic meta-analysis of systemic MSC administration in preclinical bone regeneration revealed significant increases in bone mineral density (SMD 3.02), bone volume/tissue volume (SMD 2.10), and new bone area (SMD 7.03) [129]. Additionally, both systemic and local MSC delivery enhanced healing in murine fracture models, although systemic delivery was more feasible in diffuse skeletal injuries [134].

Osteoporosis-related bone regeneration

In osteoporotic models, MSCs have demonstrated potential in restoring bone mass and microarchitecture. Transplantation of BM-MSCs in OVX-induced osteoporotic animals resulted in significant improvements in BMD, trabecular thickness, and osteogenic marker expression (ALP, osteocalcin), along with reduced TNF-α and enhanced trabecular microstructure [135]. However, systemic administration of unmodified allogeneic MSCs failed to halt bone loss in osteoporotic rats—and, in some cases, repeated injections accelerated bone loss, underscoring the need for targeted homing enhancements, such as integrin modification or scaffold-based delivery [136]. Furthermore, the regenerative capacity of MSCs in osteoporotic individuals appears preserved: MSCs derived from osteoporotic patients exhibited comparable or superior consolidation in fracture models, particularly after systemic delivery, compared to MSCs from osteoarthritic donors [137]. These findings underscore the necessity for delineated strategies local delivery may be more appropriate for isolated bone defects, while osteoporosis-related applications may benefit from engineered MSC homing mechanisms to enhance systemic targeting efficacy.

Challenges and limitations

Despite their regenerative potential, the clinical translation of MSCs for bone repair remains constrained by several key limitations (Table 2). One of the foremost issues is poor in vivo survival and engraftment, with the majority of transplanted cells failing to persist or integrate due to the harsh microenvironment of bone injury sites. Immunogenicity, particularly in allogeneic applications, poses risks of host rejection, while genetic or epigenetic modifications raise concerns about tumorigenicity [138]. MSCs surface modification protocols vary widely across laboratories, leading to inconsistency in therapeutic outcomes. From a manufacturing standpoint, challenges in large-scale expansion, maintaining phenotypic stability, and meeting Good Manufacturing Practice (GMP) requirements further complicate clinical translation [139]. Regulatory uncertainty, coupled with ethical concerns about cell sourcing and manipulation, continues to hinder standardized approval pathways [140]. Additionally, insufficient vascularization, the complexity of scaffold integration, and lack of long-term safety data limit broader adoption. Collectively, these obstacles emphasize the need for robust, reproducible, and safe manipulation strategies that align with regulatory and clinical frameworks [141].

Moreover, genetic modification of MSCs introduces unique and critical biosafety challenges. Prolonged ex vivo expansion has been shown to lead to spontaneous malignant transformation in a significant proportion of human bone marrow-derived MSC cultures 45.8% in one study resulting in tumorigenic phenotypes when transplanted into immunodeficient mice (cells formed rapidly growing lung tumors) [142]. Even in cultures without overt malignant transformation, chromosomal abnormalities including DNA copy number variations (CNVs) and aneuploidy have been detected upon long-term passaging, raising concerns over genomic stability

Table 2 Summary of major limitations associated with different types of MSC-based therapies for bone regeneration, highlighting clinical and translational barriers

Type of MSC-Based Therapy	Limitation	Description	References
Unmodified MSC Transplantation	Low in vivo survival	Transplanted MSCs exhibit poor viability due to harsh bone injury environments.	[145]
Allogeneic MSC Therapy	Immunogenicity	Risk of host immune rejection remains significant in allogeneic applications.	[146]
Gene-Modified MSCs	Tumorigenicity	Genetic manipulation may lead to uncontrolled growth or oncogenic mutations.	[147]
MSCs with Scaffold Integration	Scaffold-cell mismatch	Mechanical or biochemical incompatibility can impair MSC attachment and differentiation.	[148]
MSC Expansion on Microcarriers	Limited scalability	Difficult to scale up while preserving phenotype and osteogenic capacity.	[103]
Epigenetically Modified MSCs	Protocol variability	Variability in modification protocols affects reproducibility across labs.	[149]
MSCs with Angiogenic Preconditioning	Poor vascularization	Despite angiogenic cues, inadequate vascular support often hampers full integration.	[150]
Donor-Derived MSCs (BM, AD, UC)	Donor variability	Cell potency and immunomodulation potential vary with donor age and health.	[151]
MSC-Loaded Injectable Hydrogels	Inconsistent osteogenic differentiation	Variable responses due to material properties or local tissue conditions.	[152]
GMP-Grade MSC Expansion Systems	GMP compliance challenges	Complex documentation, traceability, and sterility requirements increase regulatory burden.	[153]
Scaffold-Free MSC Sheets	Limited engraftment	Lack of 3D support can reduce the structural integration in large defects.	[154]
Exosome-Loaded MSCs	Lack of long-term efficacy data	Therapeutic benefits are promising but long-term studies are insufficient.	[155]
Genetically Enhanced MSCs (BMP-2)	Regulatory uncertainty	No clear approval pathways for gene-edited cell therapies in most regions.	[156]
Bioactive Surface-Coated MSC Implants	High production costs	Functionalization and sterilization increase economic barriers.	[157]
MSCs from Fetal/Perinatal Sources	Ethical concerns	Use of certain cell sources may raise ethical issues regarding consent and origin.	[158]

[143]. Further compounding these risks, the introduction of immortalizing or transgene constructs such as hTERT, SV40 large T antigen, or oncogenes like c-Myc and BMI-1 is associated with potential loss of cell cycle checkpoint control, karyotypic instability, and insertional mutagenesis, all of which may drive neoplastic transformation [144]. Preclinical studies also report that murine MSCs are particularly susceptible to oncogenic transformation: passage-3 MSCs implanted into mice have developed sarcoma-like tumors, a risk that underscores the need for cautious translation of gene-modified MSCs from animal models to human clinical applications [19].

Emerging trends and future directions

Emerging directions in MSC-based bone regeneration are rapidly evolving through the integration of precision gene editing, smart biomaterials, computational biology, and personalized medicine frameworks [159]. At the forefront, gene-editing technologies such as CRISPR-Cas9, base editing, and prime editing are being harnessed to selectively enhance osteogenic gene expression in MSCs without disrupting genomic integrity. These tools allow fine-tuned regulation of transcription factors like *RUNX2* or *SP7*, as well as immune-modulatory genes to improve allogeneic cell acceptance [160]. However, these approaches are not without risks. CRISPR-Cas9 has been associated with off-target effects, where unintended genomic sites are edited, potentially leading to harmful mutations or genomic instability. Moreover, the ethical implications of human germline editing remain contentious, with concerns about informed consent, potential misuse for eugenics, and the long-term impact on human evolution. Therefore, while CRISPR-based strategies offer promising avenues for enhancing osteogenesis, their clinical application necessitates rigorous safety assessments and ethical considerations [161]. Compared to traditional viral transduction, these non-integrative systems minimize insertional mutagenesis risks, offering greater translational safety [162]. Simultaneously, artificial intelligence into stem cell engineering has transformed MSC formulation design. AI-driven models trained on multi-omics datasets—combining transcriptomic, proteomic, and metabolomic profiles can now predict MSC behavior in response to scaffold composition, bioactive loading, and environmental conditions. This capability enables the rapid development of optimized, patient-specific regenerative formulations while minimizing empirical trial-and-error [163]. In the domain of biomaterials, smart bioresponsive implants represent a significant leap forward. These platforms incorporate embedded biosensors that monitor microenvironmental parameters—such as

pH, inflammatory cytokines, or mechanical stress—and adjust the release of therapeutic agents (growth factors or anti-inflammatory molecules) accordingly. Such closed-loop systems are particularly relevant in post-traumatic or osteoporotic environments where dynamic regulation of healing is required [164]. Moreover, personalized MSC grafts is reshaping clinical delivery strategies. Using patient-derived imaging data (CT or MRI), computer-aided design and additive biomanufacturing enable the creation of anatomically tailored scaffolds loaded with tailored MSCs. Advanced bioinks incorporating osteoconductive and angiogenic cues further support spatial patterning of osteogenesis and vascular integration, especially in irregular or critical-sized bone defects [165].

On the translational front, there is increasing emphasis on multi-arm, multicenter clinical trial design to accommodate inter-patient variability and accelerate regulatory validation. Such designs allow simultaneous comparison of delivery modes, cell sources, or MSC phenotypic modulation strategies, enhancing data robustness while reducing time to market. Additionally, the development of Good Manufacturing Practice (GMP)-compliant bioprocessing platforms is critical for scalable MSC expansion and quality control, which remains a major bottleneck in clinical readiness [153]. Looking forward, integrating quantitative pharmacokinetic and pharmacodynamic (PK/PD) modeling of MSC-based therapies, which can inform dosing regimens, biodistribution, and therapeutic windows—currently underexplored in cell-based regenerative medicine [153]. Furthermore, the use of multi-omics-integrated digital twins—virtual patient-specific models predicting therapeutic outcomes may enable pre-intervention simulations to enhance treatment planning and regulatory approval processes. These innovations mark a transformative phase in MSC-mediated bone healing. The convergence of high-precision gene modulation, intelligent delivery systems, predictive modeling, and personalized bioengineering is poised to overcome longstanding translational barriers, moving MSC therapies from bench to bedside with greater efficiency, safety, and patient specificity [166].

Despite advances in MSC therapies, economic barriers limit their broad clinical adoption. High manufacturing and quality control costs hinder reimbursement and accessibility, especially in low- and middle-income countries where healthcare funding is limited. While some studies suggest MSC treatments may be cost-effective compared to standard care in specific conditions, comprehensive long-term economic data remain scarce. These financial challenges restrict MSC therapies mainly to affluent regions and specialized centers, adding to the regulatory hurdles faced globally [167, 168]. Mesenchymal stem cell (MSC) therapies face significant regulatory and standardization challenges across global jurisdictions, notably under agencies like the FDA (USA) and EMA (Europe). In the U.S., MSCs are regulated as biologics requiring Investigational New Drug (IND) applications under strict cGMP conditions, while the EMA classifies them as Advanced Therapy Medicinal Products (ATMPs), demanding complex clinical and quality documentation through centralized procedures [44, 169, 170]. However, regulatory pathways remain heterogeneous, with countries like Japan adopting conditional approval systems and others lacking robust oversight, contributing to inconsistent clinical access and proliferation of unregulated therapies [171]. A major bottleneck lies in the lack of standardized stem cell characterization current identity criteria (CD73, CD90, CD105 expression) fail to predict therapeutic efficacy, and validated potency assays (immunomodulation or angiogenesis) are not universally implemented in clinical-grade production [172]. Furthermore, reporting across clinical trials is inconsistent; many studies omit critical viability, function, or batch-specific data, limiting reproducibility [173]. Although efforts by bodies like ISO, NIST, and the Standards Coordinating Body are underway to harmonize manufacturing and quality assessment protocols, the absence of universally accepted benchmarks continues to hinder global regulatory convergence and clinical translation of MSC-based therapies [174].

Future clinical integration framework

The integration of MSC-based therapies into orthopedic clinical practice requires a structured and multifaceted approach. The first step in this framework involves scalable, GMP-compliant production of MSCs, ensuring the cells are produced at a high standard for safety, consistency, and efficacy. The need for large-scale expansion of MSCs, coupled with efficient storage and transport systems, will be essential to meet the demands of clinical applications, particularly in managing large patient populations. Once MSCs are produced, the next challenge lies in clinical translation and regulatory approval. In many countries, this requires thorough preclinical data supporting the safety and efficacy of MSC-based therapies, including robust evidence from animal models and early-phase human trials. Regulatory agencies like the FDA or EMA require clear demonstrations of cell viability, immunogenicity, and potential for tumorigenicity, which necessitates meticulous quality control protocols during production and administration. The use of standardized clinical protocols, including clear guidelines for cell dosage, administration route, and follow-up care, will be key to ensuring widespread acceptance [168, 175].

Another critical component is the training and integration of healthcare professionals, including orthopedic surgeons, cell biologists, and medical technologists, who will need to familiarize themselves with MSC therapies,

novel delivery platforms, and post-infusion care procedures. Furthermore, healthcare facilities will need to establish specialized units capable of handling the advanced nature of these treatments, including 3D bioprinting technology, bioactive scaffolds, and injectable hydrogels that support MSC function during in vivo integration. Multi-center clinical trials will be required to assess the safety and efficacy of MSC-based therapies across diverse patient populations. The clinical integration framework must account for the biological variability seen in different patient groups (e.g., age, comorbidities), and testing should include multi-arm trials designed to optimize MSC activation and delivery methods for specific subpopulations. Once clinical validation is achieved, insurance and reimbursement pathways must be defined, ensuring that these therapies are accessible to patients. The emergence of digital health tools as real-time monitoring systems and AI-driven patient stratification models could enhance clinical decision-making. These technologies would support clinicians in adjusting MSC-based treatments in response to real-time feedback from patients, thus maximizing the therapeutic outcomes. By addressing these components in a cohesive, step-by-step framework, MSC-based therapies can be effectively integrated into orthopedic clinics, providing a robust solution for bone regeneration and joint repair in patients suffering from degenerative diseases, fractures, and other musculoskeletal disorders [168].

Conclusion

The functionalization of mesenchymal stem cells presents an exciting frontier in the field of bone regeneration therapies, with the potential to revolutionize current treatment strategies for bone defects and fractures. Significant advancements have been made through various techniques such as gene modification, preconditioning, nanoparticle integration, and scaffold-based delivery systems. These methods have shown considerable promise in enhancing the osteogenic potential of MSCs, promoting bone repair, and improving overall healing outcomes. Delivery platforms have been designed to optimize cell activity, facilitate targeted release of bioactive molecules, and support sustained healing at defect sites. Moreover, while delivery platforms have shown promise in controlled release, the optimization of these systems for precise, patient-specific therapeutic outcomes remains an ongoing area of research. Looking ahead, emerging technologies, such as precision-engineered implants, 3D bioprinting, and artificial intelligence-driven design, hold great potential to address these challenges. These innovations can facilitate the development of more efficient and scalable manufacturing processes, as well as better-targeted therapies that align with the individual needs of patients. The convergence of these advancements signals a transformative shift in bone healing strategies, offering a promising future for the clinical application of MSC-based therapies. As the field progresses, continued research focusing on overcoming current limitations, such as large-scale production, immune compatibility, and precise delivery, will be essential to unlock the full therapeutic potential of MSCs and establish them as a cornerstone in regenerative medicine for bone repair.

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