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Review

REGULATION OF MESENCHYMAL STEM CELL OSTEOGENIC DIFFERENTIATION THROUGH PHYSICAL INTERVENTIONS: FROM MECHANISMS AND PARAMETER OPTIMIZATION TO CLINICAL TRANSLATION

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Abstract

Osteogenic differentiation of mesenchymal stem cells (MSCs) serves as the cornerstone of bone tissue engineering and regenerative medicine. Traditional biochemical induction methods exhibit limitations, whereas physical stimulation—as a non-invasive, precise, and controllable regulatory approach—demonstrates significant potential in guiding osteogenic differentiation of MSCs. This review comprehensively examines the biological mechanisms by which diverse physical stimuli (including mechanical forces, matrix properties, electromagnetic fields, low-intensity ultrasound, and photobiomodulation (PBM)) promote osteogenic differentiation in bone marrow-derived MSCs (BMSCs), analyzes parameter optimization strategies for multi-modal physical stimulation, and envisions the broad application prospects of intelligent and dynamic biomaterial systems in bone regeneration and tissue repair. Finally, this review proposes key directions for future research, emphasizing the importance of multifactorial synergistic regulation, intelligent precision interventions, development of non-invasive techniques, and clinical translation, aiming to provide theoretical foundations and novel insights for designing next-generation efficient and safe bone regeneration strategies.

Keywords: Mesenchymal stem cells, osteogenic differentiation, physical intervention, mechanical stimulation, signaling pathway, parameters, clinical translation.

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Introduction

Large-scale bone defects, often caused by severe trauma, tumor resection, or nonunion, present a formidable global health challenge in the field of clinical orthopedics [1]. Nonunion, representing a pathological stagnation in the fracture healing process, affects up to 5–10% of patients and often leads to long-term dysfunction, chronic pain, and substantial socioeconomic burdens [2]. Although autologous or allogeneic bone grafts remain the current clinical gold standard, they are respectively limited by donor site complications, limited availability, immune rejection, and potential disease transmission risks [3]. These inherent limitations significantly constrain their clinical efficacy, highlighting the urgent need to develop safer, more effective, and abundant sources of novel bone repair strategies.

Against this backdrop, mesenchymal stem cell (MSC)-based tissue engineering has pioneered a revolutionary pathway to address the challenge of bone regeneration [4]. The osteogenic regulatory mechanisms of MSCs have been comprehensively elucidated across molecular,

cellular, and tissue levels—ranging from intrinsic signaling pathway modulation (e.g., Wnt, bone morphogenetic protein (BMP)-2/Smad) to exosome-mediated paracrine effects and macrophage-mediated immune microenvironment optimization, as well as cutting-edge strategies like gene editing and engineered biomaterials. These advances demonstrate tremendous clinical translation potential [4–7]. Among various tissue sources, bone marrow-derived MSCs (BMSCs) have been universally regarded as the "gold standard" and core research subject in the field of bone regeneration due to their inherent osteogenic potential and the most extensive research history. However, the clinical translation of MSC therapies still faces a core bottleneck: how to ensure that implanted or endogenous MSCs can be precisely and efficiently guided toward the osteogenic lineage in complex pathological microenvironments such as nonunion (often accompanied by inflammation, poor blood supply, and mechanical instability). Traditional biochemical regulation strategies relying on exogenous growth factors offer high feasibility but frequently encounter multiple limitations, including high cost, unstable bioactivity, delivery challenges, and potential tumorigenicity. In contrast, physical interventions that mimic the *in vivo* physical microenvironment are emerging as a disruptive paradigm characterized by non-invasiveness, precise programmability, and superior cost-effectiveness.

The differentiation fate of MSCs is profoundly influenced by their physical microenvironment, encompassing factors such as matrix stiffness, micro-topography, fluid shear force, and mechanical tension. These physical cues efficiently translate into intracellular osteogenic instructions through activation of canonical signaling pathways (e.g., Wnt and BMP) and coordinated multi-level transduction systems involving integrin-cytoskeleton networks, mechanosensitive ion channels (e.g., Piezo1), and epigenetic modifications. Furthermore, they promote vascular-osteogenic coupling and optimize the osteo-immune microenvironment [8–12]. However, despite their promising potential, the clinical translation of physical interventions is hindered by challenges such as fragmented parameter optimization and unclear mechanisms. For instance, the complex interplay between stimulation parameters and cell sources, coupled with the existence of a "therapeutic window"(i.e., an optimal parameter range beyond which efficacy may be inhibited), makes parameter optimization a multidimensional challenge. Current research predominantly focuses on isolated parameters and lacks standardization, significantly impeding clinical translation [13–16]. Therefore, this review aims to systematically synthesize and analyze recent advances in physical intervention approaches for regulating osteogenic differentiation of MSCs (using BMSCs as primary models), while delving into their mechanisms of action and effective parameters. We anticipate that by integrating existing knowledge, we can establish a theoretical foundation for developing standardized regulatory protocols. This will pave the way for personalized and precise applications of physical intervention strategies, ultimately advancing bone regeneration medicine to new heights.

Core Mechanisms of Physical Interventions Affecting MSC Osteogenic Differentiation

Physical intervention strategies perceive mechanical signals through integrin-focal adhesion complexes, activating core pathways such as YAP/TAZ, TGF-β/BMP, and Wnt/β-catenin. These pathways promote osteogenic differentiation through cytoskeletal reorganization and epigenetic regulation. Additionally, paracrine signaling and immune microenvironment modulation also play a role (Fig. 1).

The "Perception-Transduction-Response" Cascade of Mechanical Signals

The response of MSCs to mechanical signals constitutes a highly integrated biological process. This cascade reaction spans from cellular membrane perception to nuclear gene expression, ultimately determining their differentiation fate toward osteogenic or adipogenic lineages. Primarily, integrins act as crucial connectors between cells and the external environment. Through the formation of focal adhesions, they convert external mechanical stimuli into intracellular biochemical signals. For instance, high-stiffness substrates activate the integrin-FAK-RhoA-ROCK signaling axis, thereby upregulating the expression of the osteogenic transcription factor runt-related transcription factor 2 (RUNX2) in MSCs. In contrast, soft substrates tend to promote adipogenic differentiation [17,38]. Simultaneously, mechanosensitive ion channels (e.g., Piezo1 and TRPV4) convert mechanical stimuli into electrochemical signals by mediating Ca^{2+} influx in response to fluid shear stress (FSS) or static compression. This process activates downstream pathways such as CaMKII and YAP/TAZ, directly regulating the expression of osteogenic markers in MSCs [18,19,29]. Furthermore, the primary cilium—another crucial mechanoreceptor—initiates osteogenic gene expression programs through elevated intra-ciliary Ca^{2+} concentration resulting from deflection under external forces like FSS [39].

These membrane-based sensing events subsequently drive dynamic reorganization of the cytoskeleton (including actin filaments, microtubules, and intermediate filaments). For instance, under cyclic tensile stimulation, Rho GTPases are activated and guide the alignment of actin stress fibers along the stretching direction. This cytoskeletal remodeling generates internal tension that is transmitted to the cell nucleus through the LINC complex, a "mechanical bridge", leading to chromatin structural rearrangement and rendering the genomic regions associated with osteogenic-related genes more accessible. Concurrently, transcription factors such as YAP/TAZ translocate into the nucleus and bind to

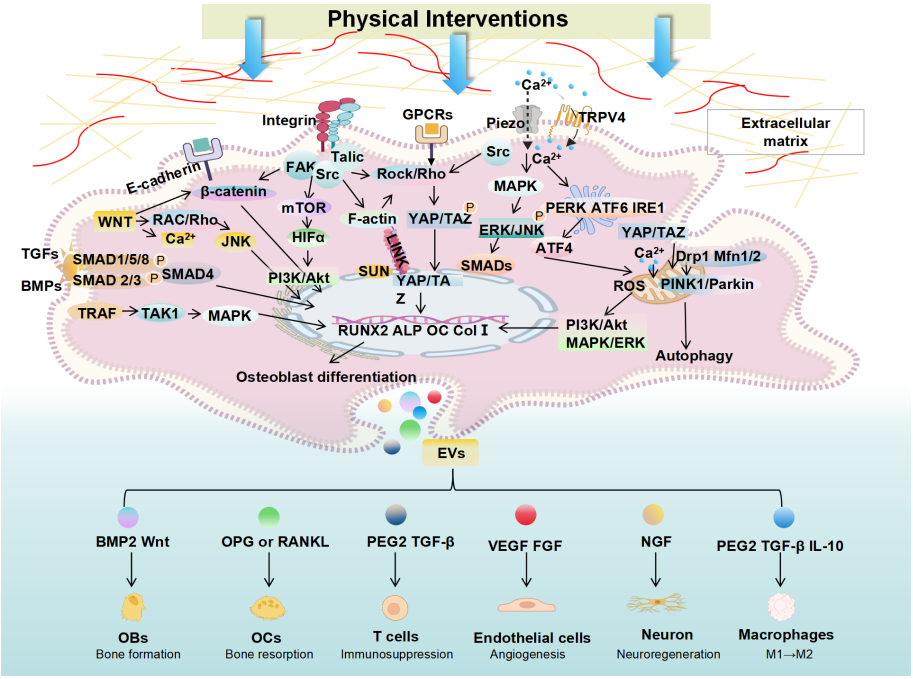

Fig. 1. Mechanisms of physical interventions affecting MSC osteogenic differentiation. Physical stimuli, including mechanical, electrical, and electromagnetic signals, are sensed by receptors such as integrins, GPCRs, ion channels, and extracellular matrix (ECM) components, thereby activating intracellular pathways including MAPK, PI3K/Akt, Wnt/β-catenin, YAP/TAZ, and Ca^{2+}-related signaling. These pathways converge on key transcription factors such as RUNX2 to promote osteogenic differentiation. MSC-derived EVs also mediate intercellular communication by delivering bioactive molecules that influence osteogenesis and the surrounding microenvironment [17–37]. Created in BioRender. Zhang, F. (2026) https://BioRender.com/r6zphl5.

promoters in these accessible regions, precisely initiating the transcriptional program of osteogenic genes [20,40–42]. In addition to F-actin, microtubules have also been shown to act as a "mechanical rheostat" for YAP/TAZ activity by regulating the stability of AMOT proteins under mechanical stimulation [43]. Notably, recent studies have proposed an "inside-out" model wherein external physical constraints directly compress the cell nucleus, increasing nuclear membrane tension. This promotes rapid nuclear translocation of YAP through conformational changes in nuclear pores, thereby initiating cellular mechanoresponse programs. This suggests nuclear deformation itself may serve as the starting point for mechanical sensing [44]. However, this sophisticated regulatory mechanism may become dysregulated under pathological conditions. For instance, aberrant matrix stiffness can drive a vicious cycle of fibrosis or tumor progression through sustained hyperactivation of integrin signaling [45,46], providing a crucial caveat for future clinical applications.

Integration and Crosstalk of Core Signaling Pathways

YAP/TAZ Pathway

As core effector molecules of the Hippo signaling pathway, YAP/TAZ serve as the central hub connecting the cellular mechanical microenvironment with the osteogenic differentiation fate of MSCs [47]. Their function originates from precise perception of physical stimulation: when mechanical signals such as matrix stiffness or tensile stress are transmitted via integrins and the cytoskeleton, they activate the RhoA-ROCK signaling pathway. This subsequently inhibits the activity of Hippo pathway kinases LATS1/2, resulting in YAP/TAZ dephosphorylation and their translocation from the cytoplasm to the nucleus [21,48]. Mechanosensitive ion channels such as TRPV4 and Piezo1 mediate calcium influx under mechanical stimulation and can also influence YAP/TAZ activity. In the nucleus, YAP/TAZ not only function as transcriptional co-activators that bind to TEAD to upregulate the expression of osteogenic-related genes (e.g., RUNX2, Osteopontin (OPN), Osteocalcin (OCN)) and promote osteogenic differentiation [22,49–51], but also serve as sophisticated signal integrators. Their activity is precisely regulated by epigenetic and post-translational modifications including phosphorylation, acetylation, and palmitoylation, and they engage in crosstalk with multiple signaling pathways such as PI3K-Akt, Wnt, and TGF-β. This ensures both long-term retention of mechanical signals and dynamic cellular responses [22–25]. It is particularly critical that YAP/TAZ holds signaling priority in mechanical responses. Through Importin-7-mediated preferential nuclear transport, they restrict or even exclude nuclear entry of other signaling molecules under specific mechani-

cal conditions, thereby ensuring the accuracy and dominance of cellular response orientation [52]. Furthermore, the YAP/TAZ-TEAD axis serves as a key "brake signal" for MSC immunomodulatory function. Inhibiting this axis activates the NF-κB signaling pathway and upregulates expression of immunoregulatory genes (e.g., TSG-6, IDO) [53].

TGF-β/BMP Pathway

The classical TGF-β/BMP signaling pathway primarily relies on the mediation of the SMAD protein family. After TGF-β or BMP ligands (e.g., BMP-2, BMP-4) bind to their receptors, they promote the formation of complexes involving phosphorylated SMAD1/5/8, phosphorylated SMAD2/3, and SMAD4. These complexes then translocate into the nucleus to initiate transcription of key osteogenic genes such as RUNX2 and Osterix [54,55]. As a crucial external signal for MSC osteogenic differentiation, mechanical stimulation activates crosstalk with pathways including PI3K/Akt, Wnt/β-catenin, MAPK family members (ERK1/2, JNK, p38), and Rho GTPases, enhancing cellular perception and response to mechanical forces [26,27]. Non-coding RNAs (e.g., miRNA-27a) also participate in BMP signaling pathway regulation, significantly enhancing stem cells' osteogenic differentiation capacity and promoting new bone formation *in vivo* [56]. Additionally, it is noteworthy that the osteogenic capacity of stem cells depends not only on their source but also on their "signal environment". Through precise modulation of the balance among BMP, TGF-β, and FGF signaling, it is possible to overcome the inherent heterogeneity between BMSCs and adipose-derived MSCs (ASCs), thereby maximizing their osteogenic potential. This approach also provides new insights for developing more efficient stem cell therapies [57].

Wnt/β-catenin Pathway

The Wnt/β-catenin pathway serves as a critical bridge linking the mechanical microenvironment to the osteogenic differentiation of MSCs [8,58]. The canonical Wnt signaling pathway activates the transcription of osteogenic genes by inhibiting β-catenin degradation and promoting its nuclear translocation. For instance, Wnt7a activates the canonical Wnt pathway, enabling its downstream transcription factor TCF1 to directly bind to the promoter region of the RUNX2 gene, thereby activating RUNX2 transcription [28]. This pathway does not operate in isolation but is precisely regulated by a multi-dimensional network. At the input level, mechanical stress promotes the phosphorylation of GSK-3β, reducing its phosphorylation and degradation of β-catenin [59]; Simultaneously, it interacts with the Wnt/β-catenin pathway by promoting intracellular calcium signaling and CaMKII activation, synergistically enhancing osteogenic differentiation [29]. Internally, molecules such as Sema3A, FOXQ1, and ZEB1 form complex positive and negative feedback loops, enabling precise signal fine-tuning [60–62]; At the systemic level, it engages in crosstalk with pathways like BMP and TGF-β, and undergoes epigenetic regulation by non-coding RNAs such as lncRNAs, thereby integrating multiple signals including mechanical, chemical, and even neural cues [63–65]. Ultimately, through this dynamic and multi-dimensional regulatory network, the Wnt/β-catenin pathway precisely converts complex microenvironmental cues into intrinsic instructions that determine the osteogenic fate of MSCs, thereby laying the theoretical foundation and identifying key targets for developing innovative therapeutic strategies for bone-related disorders.

Crosstalk Regulatory Network

Key signaling pathways including YAP/TAZ, TGF-β/BMP, and Wnt/β-catenin dynamically regulate MSC osteogenic differentiation efficiency and directionality through synergistic, antagonistic, or integrative mechanisms via cross-talk [66,67]. As core mechanosensing regulators, YAP/TAZ not only directly facilitates β-catenin nuclear translocation to enhance its transcriptional activity, but also transmits physical cues (e.g., substrate stiffness) to the Wnt pathway, collectively upregulating key osteogenic genes like RUNX2 to promote osteogenic gene expression and bone formation [66,68]. Simultaneously, the TGF-β/BMP pathway forms a positive feedback loop with YAP/TAZ to amplify signals while regulating the expression of Wnt inhibitors such as Dkk1. This mechanism limits excessive Wnt pathway activation, prevents abnormal bone formation, and maintains tissue homeostasis [54,69,70]. This ultimately establishes a signal integration platform enabling MSCs to respond to multimodal physical stimuli (e.g., mechanical stimuli, electrical stimulation (ES), low-intensity pulsed ultrasound), ensuring precise adaptive cellular responses [71–73]. Building on profound insights into this integrative mechanism, bone tissue engineering has developed innovative strategies such as combining bioactive materials with ES to synergistically activate multiple pathways [74], or utilizing conductive polymers for targeted mRNA delivery [75]. These approaches leverage the synergistic effects of physical stimulation and functional materials, driving bone tissue regeneration toward more efficient and precise directions.

Subcellular Organelle Responses and Metabolic Reprogramming

Endoplasmic Reticulum Homeostasis and Proteome Remodeling

In the physical stimulation-mediated osteogenic differentiation of MSCs, the endoplasmic reticulum senses and responds to mechanical signals through interactions with cytoskeletal components [76]. Physical stimuli (such as mechanical stretching, ultrasound, etc.) perturb intracellular calcium ion and reactive oxygen species (ROS) levels

in MSCs through ion channel proteins TRPV1 and PKD2, inducing endoplasmic reticulum stress. This effect exhibits typical dose dependency [30]. Furthermore, the endoplasmic reticulum can sense changes in the curvature of matrix pattern edges to regulate cell migration patterns [77]. Endoplasmic reticulum stress is tightly coupled with cellular metabolic states. Moderate stress activates the unfolded protein response, ensuring efficient synthesis of key bone matrix proteins. This process directly drives osteogenic gene programs through transcription factors such as ATF4, thereby promoting bone formation [78,79]. Conversely, excessive stress triggers pro-apoptotic signals, induces MSC apoptosis, inhibits their osteogenic potential, and may lead to diseases such as osteoporosis [80,81].

Mitochondrial Dynamics and Energy Metabolic Adaptation

Mitochondria serve as an integrated hub for energy metabolism, signal transduction, and quality control, with their functional remodeling being a key determinant of cellular fate. Mechanosensitive ion channels (Piezo1/TRPV4) detect ECM stiffness alterations, triggering Ca^{2+} influx that activates the cAMP/PKA pathway to enhance mitochondrial oxidative phosphorylation. This process simultaneously regulates mitochondrial fission through ERK1/2-mediated phosphorylation of DRP1 [31]. Furthermore, mechanical stimulation activates the YAP/TAZ pathway which not only inhibits DRP1 activity to promote mitochondrial fusion and optimize network morphology for accommodating dramatically increasing energy demands during MSC differentiation, but also enhances mitochondrial biogenesis via mTOR and PINK1/Parkin pathways [31,32]. Physical stimuli including mechanical traction and low-intensity laser irradiation can drive metabolic reprogramming from glycolysis toward efficient oxidative phosphorylation through AMPK/PCK2 pathways, thereby providing sufficient ATP and precursors for bone matrix synthesis [82,83]. Crucially, mitochondrially-derived ROS serve as pivotal signaling molecules: moderate ROS levels activate downstream osteogenic pathways (PI3K/Akt, MAPK/ERK) to promote bone formation, while excessive accumulation exerts inhibitory effects [84,85]. Collectively, physical stimuli precisely translate external mechanical cues into internal biochemical instructions governing MSC osteogenic fate through an integrated "mitochondrial dynamics-autophagy-metabolism-signal output" regulatory axis.

Epigenetics and Paracrine Effects

Mechanically Driven Epigenetic Reprogramming

Within the network of physical stimulation regulating osteogenic differentiation of MSCs, epigenetic mechanisms serve as the central hub connecting mechanical signals with genetic reprogramming. External physical stimuli (e.g., mechanical stretching, matrix stiffness) activate downstream pathways such as PI3K/Akt and Wnt through the continuous physical pathway of "cell membrane-cytoskeleton-nuclear skeleton", thereby regulating histone-modifying enzymes and altering chromatin accessibility to efficiently initiate transcription of osteogenic genes (e.g., RUNX2, Osterix) [33–35]. Secondly, fine regulation at the post-transcriptional level is achieved through mechanisms involving microRNA, long non-coding RNAs, etc [86]. Importantly, such mechanically induced epigenetic reprogramming establishes stable "cellular memory", which not only maintains the osteogenic state of MSCs but also optimizes the bone regeneration microenvironment by regulating their paracrine effects [87,88].

Paracrine-Mediated Microenvironmental Synergy and Transcellular Communication

Through complex intercellular communication with osteoblasts, bone cells, osteoclasts, immune cells, and vascular endothelial cells in their microenvironment, MSCs constitute the core regulatory network that maintains skeletal dynamic balance. In response to physical stimuli, paracrine signaling acts as a critical communication hub linking the mechanical microenvironment to osteoblast fate responses. Taking macrophages as an example, physical intervention not only finely regulates the dynamic transition between the pro-inflammatory M1 and anti-inflammatory M2 phenotypes but also reshapes their secretory functions. Studies have shown that mechanical stimulation can directly or indirectly via MSCs induce macrophage polarization toward the M2 phenotype through YAP/TAZ and Piezo1, thereby remodeling the osteoimmune microenvironment and promoting bone repair [89–91]. Research by Bianconi *et al.* [92] demonstrated that direct current stimulation at 100 mV/mm (1 hour daily for 3 consecutive days) not only induced the polarization of THP-1-derived M0 macrophages toward the M2 phenotype but also reprogrammed M1 macrophages, as evidenced by the upregulation of M2 markers (e.g., IL10, TGM2, CD206) alongside the downregulation of CD86 and the inhibition of IL-1β and IL-6 secretion. Furthermore, Cai *et al.* [36] found that mechanical tension induces macrophage transition to a reparative M2 phenotype via the Piezo1-p53 deacetylation axis. On the other hand, physical stimulation significantly enhances the paracrine activity of macrophages, prompting them to secrete extracellular vesicles (EVs) and soluble factors (e.g., TGF-β1) enriched with specific bioactive molecules (such as UCHL3), which promote the osteogenesis of BMSCs [36,93]. Pu *et al.* [93] discovered that mechanical force induces macrophages to secrete exosomes rich in UCHL3, which promote BMSC osteogenesis by targeting SMAD1. Huang *et al.* [94] confirmed that static topological and dynamic fluid stimuli can upregulate miR-210-3p by 10-fold in macrophage-derived exosomes via activation of the Piezo1/Ca^{2+}/YAP pathway, significantly increasing the expression of osteogenic markers such as

RUNX2, Col I, and OPN in BMSCs. Additionally, Li *et al*. [95] utilizing a Flexcell FX-5000 tension system, revealed that mechanical tension promotes Drp1-mediated mitochondrial fission in macrophages, leading to the secretion of mitochondria-containing EVs (mitochondria-containing EVs, mito-EVs). These EVs are transferred to BMSCs via CD200R-CD200 interaction, thereby significantly enhancing their osteogenic differentiation and bone formation.

Key Physical Intervention Strategies and Their Parameter Optimization

External Mechanical Stimulus

Tension Strain

Tensile strain is a critical mechanical parameter for regulating osteogenic differentiation of MSCs [96]. This process initiates at the cell-matrix interface, where mechanoreceptors such as integrins and Piezo1 are activated, triggering Ca^{2+} influx and initiating multiple downstream signaling pathways including Wnt, BMP, MAPK, and PI3K/Akt [29,97–99]. Cyclic stretching also activates YAP through the ROCK/F-actin signaling axis, thereby promoting osteogenic differentiation of MSCs [48]. These signals subsequently integrate at multiple core hubs, such as enhancing osteogenic gene expression through the AMPK/SIRT1/FoxO3a axis [96]; Alternatively, by inhibiting the core kinase LATS of the Hippo pathway, phosphorylation of YAP (S127, S397) is reduced, facilitating its nuclear translocation [48]; Moreover, it directly transports RUNX2 into the nucleus via upregulation of nuclear transport protein IPO7 to initiate transcription [41]. Additionally, tensile strain mediates post-transcriptional regulation through the lncRNA-miRNA axis (e.g., the lncRNA-MEG3/miR-140-5p axis) [100]. Notably, its effects extend beyond MSCs themselves to the microenvironment. Tensile strain activates the Piezo1 channel in macrophages, triggering deacetylation of p53 protein and inducing their polarization toward pro-repair M2-type. This promotes secretion of TGF-β1 and exosomes containing UCHL3, further enhancing MSC osteogenic differentiation [36,93]. In summary, tensile strain constructs a complex network featuring multi-level synergy—ranging from direct signal transduction to indirect microenvironmental regulation—that precisely drives the bone regeneration process.

The core of mechanical regulation for MSC osteogenic differentiation lies in the application of tension-inducing devices and techniques. These technological approaches have evolved from fundamental 2D cell stretching systems, such as applying mechanical stress to cells via elastic membranes like PDMS, to advanced platforms integrating 3D culture and microfluidics. These advanced systems can precisely simulate complex *in vivo* uniaxial/multiaxial and static/dynamic tensile environments through pneumatic, magnetic, and other actuation methods [101–104]. To ensure experimental precision, researchers not only select stretching modes based on the principle that static stretching guides morphology while dynamic stretching mimics physiological activity, but also utilize atomic force microscopy and finite element analysis to accurately characterize substrate mechanical behavior [105–107]. Currently, commercial instruments such as Flexcell and custom-built devices like iStrain collectively provide diverse tools for this field [41,108–110]. Future novel intelligent systems incorporating optical and microelectromechanical systems technologies will enable higher-precision real-time modulation and feedback control of mechanical parameters [111].

The regulation of osteogenic differentiation in MSCs by tensile stimulation constitutes a complex system collectively determined by multiple parameters including stretch amplitude, frequency, duration, and scaffold materials. Among these, stretch amplitude serves as the core variable, exhibiting an optimal osteogenic-promoting window at 5%–8% [96,112]. However, this window significantly shifts with cellular aging, as aged cells demonstrate greater vulnerability to excessive stretching and diminished responsiveness to moderate stimulation [13]. For instance, mesenchymal stromal cells derived from aged rats exhibit significantly higher apoptosis rates under excessive stretching (10%) and show poorer response to the osteogenic-promoting effect of moderate stretching (2.5%) compared to young cells [13]. Therefore, when designing intervention parameters, it is essential to optimize stimulation parameters according to cellular age to avoid cell loss and maximize therapeutic outcomes, particularly when applied to elderly patients. Regarding frequency and timing, common stretching frequencies range from 0.1 Hz to 1 Hz. Frequencies approximating circadian rhythm (e.g., 1 Hz) demonstrate superior efficacy in promoting MSC osteogenic differentiation [113,114]. Cyclic stretching outperforms static stretching. Application of 8% strain at 0.5 Hz frequency in mouse BMSCs significantly enhances alkaline phosphatase (ALP) expression and ECM mineralization [112]. Furthermore, as the scaffold materials serving as the foundation for mechanical transmission, their elastic modulus must strike a balance between transmission efficiency and biocompatibility. For example, softer substrate materials lead to dispersed stress, while stiffer materials can transmit tension more effectively but may compromise cell adhesion [115]. In conclusion, future research should focus on establishing integrated multi-parameter regulatory systems, developing smart-responsive materials, and ultimately achieving precise personalized mechanical treatment strategies tailored to special.

Hydrostatic Pressure (HP)

HP is widely used to simulate various physiological pressure-bearing environments such as joint cavities, deep skeletal tissues, vascular systems, and intra/extracellular spaces. It plays a crucial role in regulating diverse cellular behaviors including differentiation, migration, apopto-

sis, and proliferation [116,117]. The core mechanism involves activating integrins or Piezo1 on cell membranes, thereby initiating downstream signaling pathways such as Wnt/β-catenin, MAPK, and PI3K/Akt. This drives transcription factors including YAP1 and NFAT2 into the nucleus, upregulates expression of BMP-2, and directs MSCs toward osteogenic differentiation while simultaneously inhibiting adipogenic differentiation [118,119]. Furthermore, this process depends on the coordinated action of cytoskeletal structures such as intermediate filaments, as well as the fine regulation by non-coding RNAs including lncRNA-PAGBC/miR-133b/RUNX2 pathways [40,120]. More importantly, the effects of HP extend beyond the cells themselves. It drives MSCs to differentiate into vascular endothelial cells, creating a "angiogenesis-osteogenesis coupling" positive feedback loop that provides nutritional support for bone regeneration, thereby efficiently accelerating the entire bone tissue repair process [121]. Notably, undifferentiated stem cells and mature osteoblasts employ distinct mechanotransduction pathways to respond to the same mechanical stimuli, suggesting the existence of different pressure "switches" during the osteogenic differentiation of MSCs [122].

In vitro studies rely on gas- or liquid-driven specialized bioreactors to apply HP [123]. Researchers use bioreactors to precisely simulate physiological HP stimuli ranging from static to dynamic conditions through constant, cyclic, or stepwise pressure variations [124]. For instance, Henstock *et al.* [122] employed a custom pneumatic-hydrostatic bioreactor capable of fine-tuning sinusoidal pressure waveforms between 0.0001–2 Hz (or sustained pressure) and 0–280 kPa. One study utilized liquefied microcapsule environments to expose MSCs to cyclic HP at 5 or 50 MPa (three times per week at 37°C). Without osteogenic induction factors, MSC exposed to HP (50 MPa) showed significant increases in ALP and OPN activities [117]. Another study proposed a dynamic microgel platform in which single MSCs are encapsulated in ionically cross-linked cell-adhesive alginate microgels, stabilized using alginate-poly-l-lysine-alginate and calcium coating. Daily application of cyclic HP (200 kPa, 0.5 Hz) for 30 minutes demonstrated that individual MSCs can undergo complete osteogenic differentiation without biochemical induction [125].

The efficacy of HP in regulating MSC osteogenic differentiation is not determined by a single parameter, but rather by a multidimensional parameter space collectively formed by pressure amplitude, frequency, waveform, duration, and application protocol [117,122]. The pressure amplitude promoting MSC osteogenic differentiation typically ranges from 0.1 MPa to 50 MPa, with moderate intensity (e.g., 0.5–1 MPa) demonstrating remarkable effects. Beyond 100 MPa, cellular responses shift from short-term metabolic activation to long-term cellular damage [117]. Frequencies predominantly fall within 0.5–1.0 Hz to mimic circadian rhythm, while higher frequencies (e.g., 10–60 Hz) exhibit significantly weakened efficacy [122,126]. For instance, investigations into cyclic HP effects on hMSC osteogenic differentiation in 3D culture revealed that only combined stimulation at 1 Hz frequency and 280 kPa pressure could induce mineralization as high as 75% without requiring growth factors. In contrast, static culture, constant pressure (280 kPa, 0 Hz), low-frequency stimulation (0.05 Hz, 280 kPa), or low-pressure stimulation (70 kPa, 1 Hz) demonstrated no significant osteogenic effects (mineralization $< 2\%$) [122]. The effects of HP may exhibit cumulative properties; under prolonged dynamic cyclic stimulation (e.g., 1–4 hours daily for several weeks), physiological pressure levels as low as 10 kPa can drive the commitment of hBMSCs to the osteogenic lineage [127]. Studies indicate that with increasing strain amplitude, MSC differentiation shifts from osteogenic to chondrogenic, reaching peak chondrogenic-promoting effects at 15% strain; When the strain increased to 20%, this effect weakened correspondingly, and chondrogenic differentiation was significantly inhibited [15]. However, the effective thresholds of these parameters are significantly influenced by a series of biological and experimental factors. For instance, the mechanosensitivity of cells exhibits significant individualized variations depending on their tissue origin and culture medium composition [122,128]. Therefore, determining the optimal HP intervention protocol must be a comprehensive decision-making process. It necessitates systematic integration of multidimensional information including cell source, biological indicators, expression of osteogenic functional markers, and activation levels of signaling pathways to achieve precision control.

FSS

FSS, as a key physical stimulation, effectively activates the mechanical sensing mechanisms of MSCs by simulating physiological blood flow and tissue fluid dynamics. It has been demonstrated to significantly promote osteogenic differentiation both *in vitro* and *in vivo* [129–131]. This process initiates when FSS activates mechanoreceptors (e.g., integrins, primary cilium) and mechanosensitive ion channels (e.g., Piezo1/2, TRPM7) on the cell membrane, triggering intracellular calcium ion concentration changes and initiating mechanotransduction [132–134]. At the molecular level, FSS activates multiple core signaling pathways including YAP/TAZ, Notch, MAPK, and Wnt/β-catenin, upregulates the expression of osteogenic markers such as ALP, OPN, and OCN, and ultimately promotes matrix mineralization and bone-like tissue formation [69,97,132,135]. Furthermore, studies have revealed more refined regulatory mechanisms. For instance, FSS-induced autophagy regulates osteogenic differentiation through AnnexinA6, while FSS enhances the synergistic effects between MSCs and endothelial cells. Through activation of the integrin β1-FAK-ERK1/2 signaling axis, FSS further el-

evates osteogenic capacity, collectively forming an intricate network through which the mechanical microenvironment regulates bone regeneration [136,137].

In vitro simulation systems for FSS loading represent core tools for investigating MSC osteogenic differentiation, with their technological frameworks having reached considerable maturity. Primarily, these systems are categorized into four major types [138]. Parallel plate flow chambers are suitable for fundamental observations in two-dimensional homogeneous environments [139]; Cone-and-plate viscometers excel at applying adjustable uniform shear forces in three-dimensional settings [131]; and microfluidic chips enable precise simulation of complex physiological fluid environments and facilitate high-throughput monitoring [140,141]; While perfusion bioreactors integrate three-dimensional scaffolds to construct dynamic culture environments most closely resembling human physiological conditions, facilitating clinical translation [135,142,143]. Furthermore, to achieve precise control of FSS, researchers have introduced modeling methods such as computational fluid dynamics and dynamic cellular response kinetics to precisely quantify mechanical parameters within bioreactors [144]. More importantly, the combination of FSS and biomaterials, through synergistic effects, significantly enhances MSC osteogenic differentiation and substantially improves its clinical application potential in bone defect treatment [131]. Therefore, future research directions involve designing more optimized dynamic bioreactors and microfluidic systems tailored to specific application needs to maximize the promotion of MSC osteogenic differentiation, thereby laying a solid scientific foundation and technological guarantee for bone tissue engineering and clinical bone repair.

The realization of the osteogenic effect promoted by FSS highly depends on the meticulous optimization of a series of mechanical parameters, which constitutes a multidimensional and multilayered systematic project [145]. First, at the macroscopic level, there are three key parameters: in terms of strength, 1–20 dyn/cm^2 is generally considered the effective range, while 5–12 dyn/cm^2 represents the optimal interval [139]; Regarding time and frequency, intermittent stimulation mimicking physiological conditions (0.5–2 hours daily, 0.5–2 Hz) yields superior outcomes compared to continuous stimulation, effectively preventing cellular fatigue [69,131,138,146]; Regarding application modes, steady flow induces early-stage osteogenesis, whereas oscillatory flow better mimics physiological conditions to promote cellular maturation [69,130]. Secondly, at the finer micro-level, the dynamic rate and directional characteristics of mechanical stress exert decisive influences on cell fate. Research indicates that the linear increase rate of stress (ΔSS) enables precision control over differentiation orientation. For instance, when stress linearly increases from 0 to 10 dyn/cm^2, physiological slow changes (20 minutes) promote chondrogenic differentiation, moderate rapid changes (10 minutes) strongly enhance osteogenic differentiation, whereas excessive abrupt changes (0 minutes) lead to mixed differentiation [145]. Meanwhile, directional flow proves more effective than random flow in promoting osteogenic differentiation [147]. Finally, optimization of these mechanical parameters is not conducted in isolation but requires coordinated consideration with intrinsic cellular states and microenvironmental factors. This includes cell density, supplementation of growth factors (e.g., BMP-2, VEGF), and differential sensitivity to shear stress among MSCs from various sources [136,139,141]. Consequently, FSS parameter settings should not remain static but should be dynamically and personally optimized based on multidimensional information (e.g., cell type, culture conditions) and experimental feedback to maximize osteogenic differentiation (Fig. 2).

Physical Properties of Matrix

ECM Stiffness

Matrix stiffness, defined as the elastic modulus of ECM or scaffold materials, regulates MSC osteogenic differentiation by modulating cytoskeletal organization, signaling pathways, and energy metabolism [148,149]. Rigid substrates (>34 kPa) promote MSC osteogenic differentiation through activation of multiple signaling pathways including integrin-FAK-YAP/TAZ and PI3K-Akt-GSK-3β [149–152]. Concurrently, they optimize cellular energy metabolism by enhancing glycolytic flux, improving oxidative phosphorylation efficiency, and regulating mitochondrial dynamics (e.g., upregulating Mitofusin 1/2 expression while inhibiting Drp1 activity), thereby establishing the metabolic foundation for osteogenic differentiation [32,153]. Substrates with moderate stiffness (8–17 kPa) promote MSC differentiation into skeletal muscle cells, while soft substrates ($\sim$ 5 kPa) favor adipogenic differentiation. Extremely soft substrates (0.1–1 kPa) induce neural differentiation [150,154]. Notably, overexpression of miR-99b—a key mechanosensitive molecule—has been shown to overcome the limitations of soft substrates by regulating mTOR signaling, thereby promoting BMSC osteogenic differentiation within 3D soft hydrogels (3.2 kPa) [155]. Furthermore, stiffness requirements vary across bone healing stages. During early phases, MSCs respond to softer matrices via MAPK/Hippo/AP1 pathways, secreting immunomodulatory factors like TSG-6 to create a low-inflammatory microenvironment conducive to tissue regeneration [156,157]. Biomaterial design should therefore be optimized to achieve precision control of MSC osteogenic differentiation by accommodating stage-specific stiffness variations. Enhanced autophagy activity in BMSCs correlates with elevated stiffness and coincides with intensified osteogenic differentiation [157]. Conversely, lower ECM stiffness (0.7 kPa) impairs macrophage secretion and uptake functions by hindering the sorting of vesicle transport proteins and autophagy-related lipids into MSC-EVs, offer-

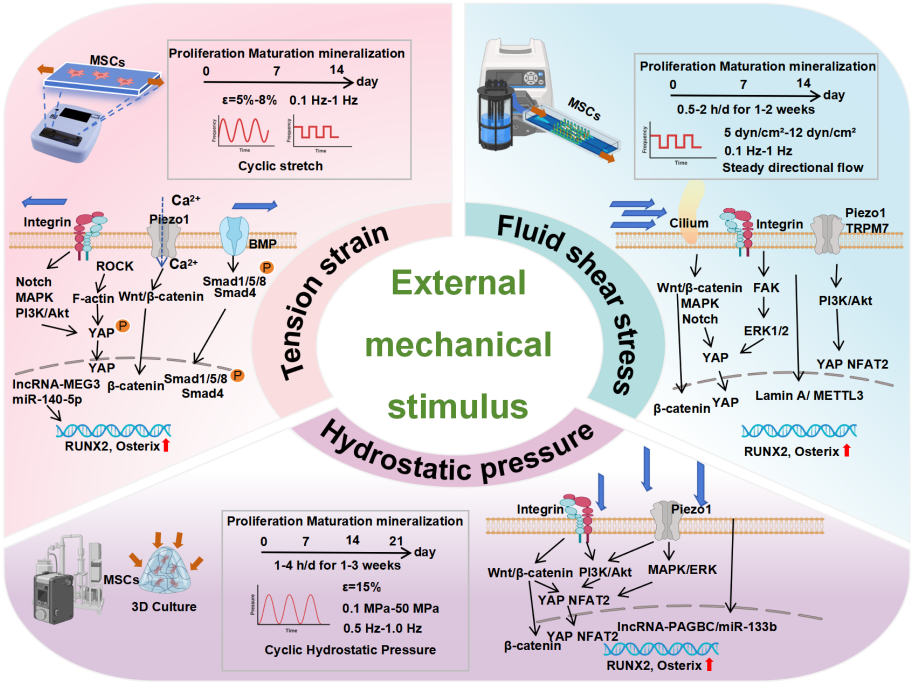

Fig. 2. External mechanical stimulus affecting MSC osteogenic differentiation. Tensile strain, HP, and FSS regulate MSC osteogenic differentiation through mechanotransduction pathways. These stimuli are sensed by mechanoreceptors such as integrins, Piezo1, and primary cilia, leading to activation of downstream signaling cascades and osteogenic transcription factors, thereby promoting osteoblast differentiation and matrix mineralization. Their effects are parameter-dependent and influenced by loading magnitude, frequency, duration, and mode [15,29,69,96–99,112–114,117–120,122,127,131–135,138,139,146]. Created in BioRender. Zhang, F. (2026) https://BioRender.com/pazii9c.

ing novel insights for designing physical stimulation-based bone repair biomaterials [158].

Precisely modulating matrix stiffness to guide the behavior of MSCs has become a key strategy in bone tissue engineering. This technology primarily relies on tunable hydrogels and micropatterned surfaces [159,160]. Hydrogels such as gelatin methacrylamide (GelMA), polyacrylamide (PAAm), polyethylene glycol (PEG), and collagen exhibit excellent biocompatibility. By adjusting cross-linking density, their stiffness can be precisely tuned within the range of several kPa to several hundred kPa [160–163]. Furthermore, hydroxyapatite nanoparticles are often incorporated to enhance mechanical strength, thereby better mimicking natural bone [164]. Micropatterned surface technology promotes spatial organization of cells and enhances cell-matrix interactions [165]. Given that the bone formation process inherently exhibits spatiotemporal characteristics with dynamically increasing matrix stiffness, research strategies have evolved from static regulation to dynamic simulation [151]. On one hand, designing fixed material stiffness gradients enables spatial directional differentiation of MSCs. On the other hand, fixed stiffness gradient scaffolds constructed using techniques like 3D printing to simulate bone structures from soft to hard can guide directional migration and differentiation of MSCs [164,166]; Utilizing smart materials to design dynamic stiffness hydrogels enables programmable enhancement of stiffness over time. For instance, Li *et al.* [151] discovered that late-stage stiffness enhancement significantly promotes bone matrix secretion and YAP/TAZ nuclear localization in MSCs. The integration of these advanced materials and technologies facilitates precise spatiotemporal control of matrix stiffness across a broad range from kilopascals to megapascals, thereby providing an ideal mechanical microenvironment for MSC osteogenic differentiation.

Matrix stiffness serves as a core mechanical microenvironmental cue regulating MSC osteogenic differentiation, with its regulatory effects being finely influenced by multidimensional parameters. Studies indicate that an elastic modulus range of 60–140 kPa (with approximately 60 kPa showing particularly prominent effects) can significantly induce MSCs to adopt a stellate morphology and upregulate osteogenic gene expression [167]. The nanoscale homogeneity of the matrix is equally critical. Homogeneous hydrogels better promote osteogenic differentiation [168] by forming more effective focal adhesions and actin bundles. The intrinsic cellular conditions and dynamic responses constitute another determining factor. MSCs from different sources (e.g., bone marrow and adipose-derived) exhibit fundamental differences in mechanical signaling perception. This necessitates stiffness parameter optimization for specific cell types to achieve precision control

[14,122]. Concurrently, cell density influences differentiation through dual mechanisms. High-density culture can directly enhance intercellular signaling and upregulate osteogenic factors such as RUNX2 [156]; It can also modulate cellular sensitivity to matrix stiffness and cytoskeletal reorganization, indirectly influencing mechanotransduction [169]. Furthermore, dynamically changing mechanical environments demonstrate greater physiological relevance than static stiffness. Studies confirm that the "soft-to-hard" dynamic stiffness transition, which simulates the *in vivo* healing process, efficiently promotes MSC osteogenic differentiation by activating the integrin-FAK signaling pathway, highlighting the importance of stiffness transition timing [151,163]. In summary, the regulation of matrix stiffness on MSC osteogenic differentiation constitutes an intricate network involving material physical properties, intrinsic cellular attributes, and dynamic mechanical temporal sequences. In the future, personalized material design integrating patient-specific biomechanical environments with cellular requirements will emerge as an indispensable and pivotal strategy in the field of bone repair and regenerative medicine.

Extracellular Fluid Viscosity

Extracellular fluid viscosity, as a crucial physical microenvironmental signal, plays a decisive role in MSC differentiation by modulating cellular mechanosensation. Research indicates that high-viscosity environments can effectively promote osteogenic and chondrogenic differentiation while inhibiting adipogenic differentiation in MSCs through increased mechanical loading; Conversely, low viscosity favors adipogenic differentiation [170,171]. Regarding osteogenic differentiation, high viscosity induces the formation of dense Arp2/3-dependent actin networks and elevates membrane tension through NHE1-dependent cell swelling and ILK-mediated cell spreading. This ultimately activates the TRPV4 ion channel, triggering calcium influx. Subsequently, two parallel pathways coordinately regulate gene expression: First, calcium signaling activates the RhoA/ROCK pathway, driving nuclear translocation of YAP; On the other hand, it directly promotes the nuclear translocation of the transcription factor NFATc1. The synergistic action of YAP and NFATc1 significantly upregulates the expression of osteogenic-related genes such as ALP and RUNX2, efficiently driving osteogenic lineage differentiation of hMSCs [66,172]. Notably, the effect of high-viscosity signaling is potent enough to reverse the inhibitory effects of soft substrates on osteogenic differentiation, achieving lineage conversion from adipogenic to osteogenic [66]. Furthermore, this regulatory network extends into the immune domain, where hMSCs cultured on soft substrates under high-viscosity conditions demonstrate immunomodulatory properties by promoting M2 macrophage polarization, establishing a bidirectional regulatory network connecting osteogenic and immune microenvironments [66].

Regulating extracellular fluid viscosity is an emerging strategy for promoting bone repair, which also drives the development of biomimetic materials. Methodologically, researchers have developed various models and techniques to accurately quantify viscosity. Lu *et al*. [170] constructed culture media with varying viscosities (88.8–645.5 cP) using biologically inert PEG. These were co-encapsulated with cells in agarose hydrogels to systematically analyze viscosity effects on hMSCs' trilineage differentiation. Regarding viscosity measurement techniques, beyond traditional creep testing, cutting-edge research employs new technologies such as holographic imaging and laser speckle contrast imaging, enabling non-invasive measurement of tissue models [66,173]. In terms of clinical translation prospects, regulating extracellular fluid viscosity opens new avenues for bone regeneration. By optimizing the viscosity of culture media or biomaterials, the osteogenic capacity of stem cells can be effectively enhanced, improving repair outcomes. Additionally, as a crucial biophysical parameter, abnormal changes in viscosity may serve as novel targets for diagnosing or predicting osteogenic dysfunction, supporting personalized medicine. However, clinical translation of this strategy still faces multiple challenges, including material biocompatibility, precision control of *in vivo* viscosity, and interference from complex physiological environments; these issues require urgent resolution.

Studies have shown that in two-dimensional (2D) culture, a high-viscosity environment can significantly enhance osteogenic differentiation of hMSCs while inhibiting their adipogenic differentiation by promoting nuclear translocation of YAP and β-catenin [170]. This regulation becomes more complex in three-dimensional (3D) culture systems. Dynamic viscoelastic hydrogels with rapid stress relaxation properties synergize with moderate (1.5%) compressive strain to effectively accelerate bone regeneration in rat femoral defect models [174]. Moreover, on softer matrices (~9 kPa), although cells primarily undergo adipogenic differentiation, rapid relaxation similarly suppresses adipogenesis [171]. These findings reveal that when designing 3D hydrogels for bone tissue engineering, optimizing the stress relaxation rate is equally important, if not more critical, than setting the initial stiffness [167]. Furthermore, the morphology of the cells themselves is a key factor regulating their response to viscosity. Specifically, high-viscosity environments tend to promote osteogenic differentiation while suppressing adipogenic differentiation in large and elongated hMSCs—an effect potentially associated with actin filament organization; However, viscosity does not significantly affect smaller cells [175]. In summary, extracellular fluid viscosity provides a novel strategy for advancing bone tissue engineering and bone repair. However, current related technologies remain in the laboratory stage; their clinical translation still requires overcom-

ing challenges such as material safety, precision of viscosity regulation, and the influence of complex *in vivo* microenvironments.

Surface Morphology and Topographic Structure

Matrix surface micropatterning refers to the fabrication of specific micro-to-nanoscale structures on cell culture substrates through physical or chemical methods. This technique simulates the natural cellular microenvironment and exerts significant effects on cellular behavior [51]. The core mechanism is an outside-in mechanotransduction cascade: surface micropatterns on the matrix first form specific focal adhesions by altering the spatial distribution of cell adhesion points. This regulates cell morphology and cytoskeletal reorganization, thereby remodeling the tension state of the cytoskeleton. This tension activates key signaling pathways such as RhoA/ROCK, MAPK, and Wnt/β-catenin, ultimately converging mechanical effects on the cell nucleus. This causes nuclear deformation and drives nuclear translocation of key transcription factors like YAP, inducing the expression of osteogenic-related genes such as RUNX2 and OPN [176–179]. For instance, triangular micropatterns on matrices upregulate Piezo1 and YAP1 nuclear translocation, inducing osteogenic differentiation in aged rat BMSCs [180]. Large nanospacing (>70 nm) between adhesive peptide sequences like RGD promotes integrin cluster disassembly, increases cytoplasmic actin dynamics, and facilitates globular actin translocation into the nucleus, where it polymerizes into filamentous structures. This increases nuclear tension, promoting chromatin remodeling and accessibility, which releases YAP from the SWI/SNF complex, consequently activating osteogenic genes. The pore size of biomaterial scaffolds (through its physical property "pore curvature") determines whether MSCs maintain stemness or undergo osteogenic differentiation by modulating the YAP/TAZ signaling pathway [181]. Furthermore, matrix topography is closely linked to intercellular communication. Specific spatial distributions (e.g., an MSC-to-macrophage ratio of 2:1) can guide macrophage polarization towards the M2 phenotype and induce the release of factors like Oncostatin M. These factors synergistically promote osteogenic differentiation and bone regeneration through multiple signaling pathways, including BMP-Smad and Wnt/β-catenin [182].

The core strategy for effectively regulating the osteogenic differentiation of MSCs lies in designing and fabricating matrix topography from micro to macro scales and from static to dynamic paradigms. This strategy is broadly categorized into two aspects: static topography construction and dynamic topography modulation. Static techniques provide cells with initial signals through fixed physical features. For instance, technologies such as photolithography, electrospinning, self-assembly, and 3D printing can construct biomimetic structures at micro- and nano-scales, which guide cell adhesion, spreading, and osteogenic differentiation [177,183–186]. However, static topographies cannot simulate the dynamically changing microenvironment *in vivo*, prompting the emergence of dynamic topography modulation technologies [187,188]. This technology utilizes smart materials (e.g., shape-memory polymers, responsive hydrogels) and advanced methods (e.g., acoustic tweezers) to achieve temporal and reversible changes in substrate topography or mechanical properties, thereby enabling non-invasive modulation of cellular behavior through spatiotemporally precise simulation of physiological conditions [189–191]. In summary, dynamic modulation strategies incorporating smart materials provide robust support for achieving precision bone regeneration by meticulously simulating the endogenous microenvironment.

Cell morphology serves as a crucial physical factor regulating the lineage differentiation of MSCs, with its underlying mechanisms operating across both two-dimensional planes and three-dimensional spatial contexts. On two-dimensional surfaces, the geometric morphology of cells coupled with their spreading area collectively determine their differentiation fate. Macroscopically, high contractility-inducing shapes (e.g., rectangles with aspect ratio (AR) = 2, star-shapes) promote osteogenic differentiation, whereas low contractility-inducing shapes (e.g., AR = 1 squares, circles) facilitate adipogenic differentiation [178,180,192,193]. Triangular micropatterns arranged linearly (side length: 15 μm) significantly induced cellular elongation and thinning, markedly promoting osteogenic differentiation. This was evidenced by increased expression of osteogenic genes (e.g., RUNX2, Spp1, Alpl, Bglap, Col1a1) and decreased expression of adipogenic genes (e.g., Pparg, Cebpa, Fabp4). Conversely, circular micropatterns coated with fibronectin inhibited osteogenic and adipogenic differentiation [180]. Microscopically, cell spreading area positively correlated with osteogenic differentiation, with smaller micropattern islands (e.g., 314 μm^2) promoting adipogenic differentiation, while larger islands (e.g., 1256 μm^2) significantly elevated the expression of osteogenic markers such as ALP and Col I [179]. Pattern height also modulated differentiation in a size-dependent manner; for instance, 15 nm-high patterns enhanced the expression of bone maturation markers more effectively than 8 nm-high ones [194]. Additionally, the micro-topography of surfaces proved critical, where specific roughness (Ra 0.77–3.1 μm) and groove/pillar dimensions (groove width: 4–60 μm, ridge width: <10 μm) could effectively enhance cell adhesion and cytoskeletal tension, thereby promoting osteogenesis [195–198]. In three-dimensional space, the porous structure of scaffolds, including pore size, porosity, pore interconnectivity, and pore shape, serves as the core regulator for cell infiltration, proliferation, and differentiation [190]. An ideal scaffold must biomimic the hierarchical architecture of natural bone to accommodate the distinct requirements of cancellous bone (50%–90% porosity, 50 μm to 1 mm pore size) and cortical bone (5%–30%

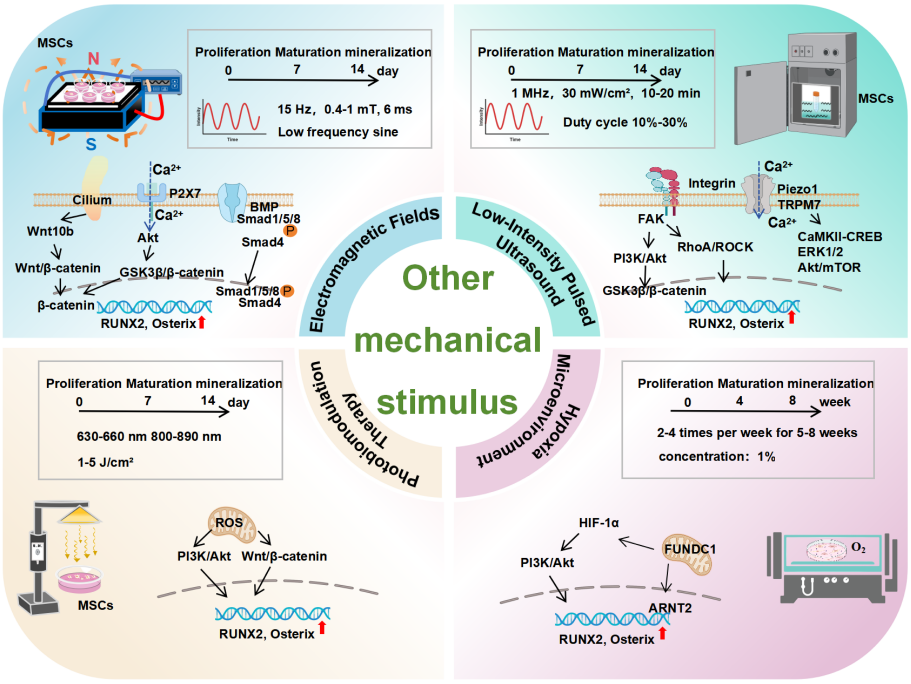

Fig. 3. Physical properties of matrix affecting MSC osteogenic differentiation. Matrix-related physical cues, including ECM stiffness, extracellular fluid viscosity, and surface morphology/topographic structure, regulate MSC osteogenic differentiation through mechanotransduction. These cues modulate cell adhesion, cytoskeletal organization, membrane tension, and downstream signaling pathways, ultimately activating osteogenic transcription factors such as RUNX2 and Osterix to promote osteoblast differentiation and matrix mineralization [32,149–152,154–157,167,171,172,174,176–180,192–198]. Created in BioRender. Zhang, F. (2026) https://BioRender.com/m5wlxhc.

porosity, approximately 1 μm to 10 μm pore size) [199]. Research indicates that the osteogenic effect relies on a complex balance between pore size and porosity. For instance, within the optimal pore size window of 600–700 μm, higher porosity (70%–90%) significantly enhances osteogenesis compared to lower porosity (40%) [200]. Pore shape can also finely regulate differentiation; for instance, rhombic pores facilitate osteogenic differentiation, whereas square pores tend to promote chondrogenic differentiation [201]. In summary, precise control over the differentiation fate of MSCs can be achieved through meticulous design of material geometry, dimensions, and surface topology. Future research directions focus on multi-parameter synergistic optimization and integration with advanced manufacturing technologies like 3D printing to achieve highly customized scaffold structures and functions, thereby meeting individualized therapeutic needs (Fig. 3)

Other Physical Microenvironmental Factors

Electromagnetic Fields (EMFs)

As a safe, non-invasive physical intervention approach, EMFs can effectively promote stem cell osteogenic differentiation and bone regeneration through precision control of parameters such as frequency, strength, and waveform. This technology has been approved by the U.S. FDA for the treatment of fracture nonunion [202]. EMFs regulate the osteogenic differentiation of MSCs through cascade reactions. EMF signals are transmitted into cells via primary cilia, activating the Wnt10b-Wnt/β-catenin signaling axis to increase peak bone mass in growing rats [203]; and promote Ca^{2+} influx by activating P2X7 receptors, enhance P2X7 expression, and activate the Akt/GSK3β/β-catenin pathway to drive osteogenic differentiation of BMSCs [72]. Meanwhile, EMF stimulation significantly promotes the expression of osteogenesis-related proteins RUNX2, OPN, OCN, and ALP by activating key signaling pathways such as BMP/Smad [204,205], and enhances osteogenic differentiation of bone marrow MSCs through regulation of the miR-34b-5p/STAC2 pathway [206]. Pulsed electromagnetic fields (PEMFs) can also synergize with biochemical signals such as BMP-2 to enhance the gene expression of BMP-2, BMP-6, and their receptors [207]. Furthermore, EMFs regulate autophagy and inflammatory responses in BMSCs while optimizing cytokine secretion, thereby promoting angiogenesis and osteoimmunomodulation more effectively [205,208] Microwave EMFs penetrate deep into tissues, simultaneously promoting osteogenesis and modulating immunity, offering new therapeutic approaches for complex bone diseases like osteomyelitis [209].

The application of EMFs in promoting MSC osteogenic differentiation is advancing into a new phase

of precision treatment featuring interdisciplinary integration with materials science, genetic engineering, and other fields. Fundamental EMF equipment (such as PEMF, Extremely Low-Frequency Electromagnetic Fields (ELF-EMFs), and radiofrequency electromagnetic fields (RF-EMFs)) can precisely control magnetic parameters to meet experimental requirements. As a low-frequency (1–300 Hz) non-continuous signal, PEMF effectively directly activates intracellular signaling pathways or synergizes with growth factors such as BMP-2 to induce osteogenic differentiation in MSCs from various sources (e.g., bone marrow and adipose-derived) [207,210]. Researchers developed a dynamic pulsed electromagnetic fields (D-PEMFs) bioreactor by combining wavelike rocking motion with pEMF. This system enables large-scale, efficient cultivation of hBMSCs while enhancing their osteogenic transcription factor activity [211]. LF-EMF can deeply stimulate bone tissue but often requires combination with biochemical factors (e.g., β-glycerol trimethylamine) for synergistic effects [212]. Although RF-EMFs offer portability and rapid application, they demand extremely stringent safety protocols. EMFs are advancing toward intelligent and personalized therapeutics through integration with technologies such as 3D printing, magnetic nanoparticles, and gene delivery [213–215]. For instance, 3D-printed titanium alloy scaffolds combined with pEMF stimulation significantly enhance the osteogenic capacity of stem cells in osteoporosis patients [213]. In the future, with the integration of materials science, nanotechnology, and electromagnetic biology, bone tissue engineering based on 3D-printed scaffolds and EMFs will become more intelligent, personalized, and efficient, providing innovative solutions for bone defect repair.

The impact of EMFs on the osteogenic differentiation of MSCs is regulated by multiple key factors, among which frequency, strength, and exposure duration constitute the central parameters. The effects of EMFs exhibit optimal frequency and strength "windows" [216]. Low-frequency sinusoidal EMFs (15 Hz, 0.4–1 mT) can effectively promote the proliferation and osteogenic differentiation of rat bone marrow MSCs [206]. EMFs (15 Hz, 1 mT) enhance osteogenic differentiation of cells implanted in HAC scaffolds [204]. Sinusoidal EMFs (50 Hz, 0.4 mT) promote self-renewal and osteogenic effects in senescent BMSCs [208]. The effects of EMFs are time-dependent [214]. For example, brief single session of low-amplitude pEMF exposure (15 Hz, 2 mT, 6 ms) proves most effective in stimulating chondrogenesis of MSCs [217]. Optimal parameters for EMFs are influenced by cell source, age, and environmental conditions [212,218,219]. Wang *et al.* [218] discovered that pEMFs can stimulate sensory nerves to produce Sema3A, significantly improving the volume fraction and plate trabecular structure of distal femoral cancellous bone in aged mice while exerting limited effects on young mice. This suggests the age-selective "on-demand efficacy" of PEMFs. In conclusion, future research should comprehensively consider parameters such as strength, frequency, waveform, duration, and cellular state. By integrating techniques like response surface analysis and machine learning, we will more efficiently identify optimal parameter combinations for specific clinical scenarios, ultimately achieving truly personalized therapeutic interventions.

Low-Intensity Pulsed Ultrasound (LIPUS)

LIPUS is a non-thermal form of ultrasonic therapy, typically characterized by a frequency of 0.7–3 MHz, intensity < 3 W/cm^2, and pulsed wave output [218]. It exerts potent effects through a multi-layered regulatory network: Primarily, at the core mechanobiological level, LIPUS utilizes mechanical force transduction, cavitation effects, and microstreaming effects to directly activate integrin α11 (ITGA11), thereby enhancing FAK/PI3K/Akt/GSK3β/β-catenin signaling pathway activity [220], or mechanosensitive ion channels (such as Piezo1, TRPM7), leading to calcium influx. This subsequently triggers key signaling pathways including CaMKII-CREB, ERK1/2, and Akt/mTOR, upregulating the expression of osteogenic genes like RUNX2 and BMP-2. These mechanisms directly drive the proliferation, migration, and matrix mineralization of BMSCs [221–223]. LIPUS also significantly enhances ALP expression and mineralized nodule formation in rat BMSCs by upregulating ITGA11 and activating the FAK/PI3K/ Akt/GSK3β/β-catenin signaling pathway [220]. At the microenvironment level, LIPUS achieves systemic regulation through multiple signaling pathways. LIPUS optimizes the immune microenvironment by promoting macrophage transformation toward a reparative phenotype via specific pathways such as PI3K/Akt-COX-2/PGE2 activation [224]; It maintains bone metabolic balance by coupling osteoblasts with osteoclasts through the EphrinB2/EphB4 signaling pathway [225]; It can also repair radiation damage by regulating the RhoA/ROCK signaling pathway [226], and when combined with stem cell therapy, it promotes vascular and neural regeneration to achieve functional tissue integration [227]. Notably, LIPUS modulates MSCs to secrete exosomes, amplifying its therapeutic effects at distal sites through this intercellular communication medium [74]. Thus, LIPUS serves as a multifunctional physical platform integrating direct cellular activation with microenvironmental optimization, holding tremendous potential in the field of bone regeneration.

LIPUS is evolving from a single stimulus to multimodal integration with biomaterials, genetic engineering, and other technologies to synergistically enhance its efficacy in bone repair [228]. LIPUS can efficiently deliver osteogenic genes (e.g., BMP-2) or genetic drugs inhibiting bone resorption (e.g., CTSK siRNA) through cavitation effects, thereby achieving precision control over the osteogenic differentiation of MSCs. This offers novel therapeutic strategies for metabolic bone diseases such as Osteo-

porosis [229,230]. The combination of LIPUS with biomaterial scaffolds like PLGA/α-TCP significantly enhances the proliferation, adhesion, and osteogenic differentiation of MSCs on scaffolds (e.g., elevating ALP activity, type I collagen expression, and calcium deposition) [231]. When combined with nanotechnology, the application of LIPUS achieves dual expansion: functionalized nanobubbles serve as "nanomechanical force generators" to amplify its biological effects. On the other hand, LIPUS can precisely trigger responsive nanosystems (such as drug-loaded nanobubbles) to release pharmaceuticals, enabling synergistic therapy of anti-infection and bone repair promotion [224]. In summary, LIPUS has evolved into a multifunctional biophysical platform that systematically enhances bone regeneration at multiple levels through integration with gene, material, and nanotechnology.

The biological effects and therapeutic safety of LIPUS critically depend on precise parameter settings—a complex system involving multivariable synergistic interactions. Output strength is pivotal for treatment and must balance effectiveness with safety. The effective intensity of LIPUS varies from tens to hundreds of mW/cm^2 depending on cells and therapeutic goals, with 30 mW/cm^2 being a commonly used reference value for bone repair [232,233]. Frequency selection primarily depends on tissue penetration and focusing capabilities of acoustic waves. 1 MHz is commonly used in bone regeneration research, while fields such as neuromodulation typically operate within the 0.3–1.5 MHz range [224,234]. Duty cycle (DC) (typically 10%–30%) regulates average energy delivery and thermal effects. The 20%–30% range has been found to significantly promote neural and bone cells, effectively activating key signaling pathways such as ERK1/2 and PI3K-Akt [235,236]. Studies have found that compared with traditional long-term low-frequency stimulation, short-term high-frequency (10 MHz, 10 min/d for 5 days) mechanical stimulation can more efficiently and rapidly induce osteogenic differentiation of human MSCs by activating piezo channels and the ROCK signaling pathway [237]. Additionally, pulse frequency (approximately 1 kHz) and pulse width collectively determine stimulation timing. The pulse frequency primarily maintains intracellular Ca^{2+} influx, while the pulse width prevents thermal effect accumulation [238]. A therapeutic window (10–20 minutes) also exists between treatment duration and cumulative energy [239]. In summary, the application of LIPUS requires comprehensive optimization of multiple parameters such as strength and frequency [232,236,240,241]. Future research should focus on elucidating the interactions between parameters and establishing standardized protocols for different therapeutic objectives to advance its precise clinical translation.

Photobiomodulation (PBM) Therapy

PBM therapy is an innovative treatment strategy that modulates cellular functions in a non-invasive, non-thermal manner, with primary wavelength ranges of 630–660 nm and 800–890 nm relevant to stem cells [242]. The core mechanism lies in photons being absorbed by cytochrome c oxidase within mitochondria, thereby activating the electron transport chain, enhancing ATP synthesis, and triggering a downstream cascade of signaling reactions that promote tissue repair [243,244]. In promoting osteogenic differentiation, PBM operates through a multi-level, synergistic regulatory network. Firstly, it directly activates two core osteogenic pathways—PI3K/Akt and Wnt/β-catenin—upregulating key gene expressions such as RUNX2 and ALP while promoting mineralization. The crosstalk between Akt and Wnt pathways forms a positive feedback loop, amplifying the osteogenic effect [12]. Secondly, PBM induces a benign oxidative stress by moderately increasing intracellular ROS levels, enabling ROS to act as crucial secondary messengers that further activate signaling pathways like Akt [245,246]. Additionally, studies confirm PBM promotes osteogenic differentiation by activating cellular autophagy [82]. Beyond direct effects on stem cells, PBM indirectly promotes bone repair by optimizing the regenerative microenvironment—for instance, promoting macrophage polarization to the anti-inflammatory M2 phenotype and upregulating pro-angiogenic factors like VEGF secreted by MSCs [247, 248]. In summary, with its non-invasive nature and minimal side effects, PBM demonstrates significant potential in tissue engineering and regenerative medicine, emerging as a prospective non-pharmacological therapeutic strategy [249,250].

The application of PBM therapy in promoting osteogenic differentiation of MSCs has evolved from a single physical stimulation into a comprehensive therapeutic approach. The therapeutic efficacy fundamentally relies on appropriate selection of light sources such as lasers and light-emitting diodes (LEDs), where lasers provide precise focusing suitable for deep tissues, while LEDs offer cost-effective and portable solutions for superficial tissues [251,252]. The effectiveness of PBM has been thoroughly validated across multiple sources of MSCs in both preclinical and clinical studies addressing conditions such as bone defects, osteoporosis, and other conditions. It exhibits unique advantages in restoring the impaired osteogenic capacity of MSCs within metabolic disorders like diabetes [82,253]. Currently, the development trend is shifting from single-factor stimulation to multifactorial synergistic enhancement [246] Notably, inspired by diatom structures, Li *et al*. [254] constructed multifunctional scaffolds with biomimetic "cytoskeleton-chloroplast-exoskeleton" architecture. Through near-infrared light triggering, the synergistic photothermal effect between Fe(Cu)OOH nanoparticles and the SiO_2 protective layer eliminated ROS, pro-

moted M2 polarization of macrophages, and activated the TGF-β signaling pathway via mild thermal stimulation and ion release. This ultimately synergistically promoted vascularized bone regeneration for infected bone defects. Moreover, AI-driven real-time image analysis technology is providing data support for optimizing PBM parameters and achieving precision clinical treatments [255,256]. Looking ahead, through establishing standardized parameter systems, exploring optimal synergistic protocols, and deepening convergence with fields such as AI and nanomaterials, PBM is poised to play a more central role in bone tissue engineering and clinical orthopedic therapies.

PBM parameter optimization constitutes a systematic endeavor: wavelength determines target sites and penetration depth, energy density governs stimulus strength and direction, while irradiation duration and frequency regulate stimulus rhythm and cumulative effects [256–259]. Red light (630–660 nm) and near-infrared light (800–890 nm) are widely used to promote cell proliferation and anti-inflammatory effects due to their strong tissue penetration capabilities, while multicolor light sources such as green and blue light can also effectively enhance the expression of osteogenic genes [257,260,261]. The efficacy and mechanisms vary across different wavelengths. For instance, 635 nm red light upregulates OPN at 2 J/cm^2 but downregulates OCN and OPN at 4 J/cm^2 [258]. Near-infrared light such as 808 nm typically promotes the expression of key osteogenic genes including RUNX2 and OCN at higher energy densities (4-5 J/cm^2) [262]. An energy density of 2-6 J/cm^2 has been identified as an effective therapeutic window for promoting osteogenic differentiation, significantly enhancing the expression of key genes such as RUNX2 and OCN [262,263]. Extremely low energy density (1 J/cm^2), when combined with photosensitizers, can achieve effective osteogenic induction [264]. Conversely, excessive doses (e.g., 16 J/cm^2) produce inhibitory effects [265]. Regular repeated irradiation (e.g., at 72-hour intervals) proves more effective than single exposures in sustaining osteogenic signaling activation, particularly under pathological conditions such as diabetes [253,259]. Furthermore, continuous wave and overlapping irradiation modes have demonstrated superior efficacy over pulsed modes in promoting MSC proliferation and osteogenic differentiation [256,266]. In conclusion, future research should focus on elucidating the synergistic or antagonistic effects of different parameter combinations. This will enable the establishment of standardized, individualized PBM treatment protocols tailored to specific cell types, pathological conditions, and therapeutic objectives, ultimately advancing precision clinical translation in the field of bone regeneration.

Hypoxia Microenvironment

The hypoxic microenvironment (1%–5% O_2), an inherent physiological feature of bone tissue, precisely regulates bone homeostasis through a complex multi-level network centered on the HIF-1α hub [267]. The stabilization and activation of HIF-1α not only directly upregulates osteogenic key genes such as RUNX2 and ALP to enhance matrix mineralization but also achieves synergistic coupling of angiogenesis and osteogenesis through VEGF induction [268–270]. In this process, HIF-1α does not act in isolation; instead, it forms a sophisticated signaling axis with key pathways including PI3K/Akt to collectively drive osteogenesis. Furthermore, this regulation extends to more refined levels: hypoxic conditions can induce FUNDC1-mediated mitochondrial fission to enhance HIF-1α activity [271] and promote osteogenesis via COX-2/PGE2/VEGF communication between endothelial cells and MSCs [272]. Concurrently, non-coding RNAs (e.g., tsRNA) and transcription factors (e.g., ARNT2) serve as precise modulators [273,274], integrating multiple dimensions—from metabolic reprogramming to organelle dynamics, and from signaling pathway crosstalk to microenvironmental communication—ultimately determining the osteogenic fate of MSCs with high efficiency.

In recent years, researchers have established a comprehensive hypoxia regulation technology system spanning from precise *in vitro* simulation to active *in vivo* intervention. In terms of *in vitro* simulation, technological advancements have progressed from single, static hypoxia environments to high-throughput, dynamic, and spatially heterogeneous precision simulation. The new generation of intelligent portable hypoxia chambers enables fine oxygen concentration regulation (1%–20% O_2 concentration, adjustable in 0.5% increments) and long-term stable maintenance [275]; To simulate the heterogeneity of *in vivo* oxygen distribution, tri-zone oxygen-controlled chambers and microfluidic-based BLOCCs chips facilitate multi-concentration parallel studies and three-dimensional linear oxygen gradient construction, respectively [276,277]. The integration of 3D printing with microfluidics has further enabled high-throughput screening platforms capable of providing 12 oxygen concentrations simultaneously in 96-well plates, significantly accelerating condition optimization [278]. Unlike *in vitro* simulation, *in vivo* intervention strategies focus on actively constructing or regulating the microenvironment at bone defect sites, transforming hypoxia regulation from “passive adaptation” to “active intervention” [279]. This is primarily achieved through three categories of biomaterials: (1) Hypoxia-responsive materials loaded with hypoxia mimetics or cobalt ion-releasing compounds that stably activate the HIF-1α pathway while synergistically promoting osteogenesis and angiogenesis [280]; (2) Oxygen-generating materials containing components like MgO_2, which provide sustained oxygen supply to address severe hypoxia at the center of large bone defects, improving cell survival [281]; and (3) Multifunctional composite hydrogels, piezoelectric materials, etc. These materials not only regulate hypoxia but also integrate multiple functions such as ROS scavenging, antibacterial activity,

and ES, creating superior protective microenvironments for MSC osteogenic differentiation. Collectively, they provide more effective therapeutic tools for bone tissue engineering [282,283].

The impact of hypoxic environments on the osteogenic differentiation of MSCs is a highly complex nonlinear process. Its effects are not solely determined by oxygen concentration but are collectively shaped by cellular intrinsic properties, external intervention strategies, and dynamic *in vivo* microenvironments. MSCs derived from different sources exhibit significant variations in oxygen concentration tolerance thresholds and osteogenic responses, with cell passage numbers and cycle status profoundly influencing their sensitivity. For instance, human periodontal ligament cells maintain stem cell characteristics effectively under specific chemically simulated hypoxia, while bone marrow MSCs demonstrate more pronounced osteogenic promotion at 1% oxygen concentration. Although adipose- and umbilical cord-derived MSCs exhibit relatively weaker osteogenic potential, it can still be improved through targeted hypoxic modulation [284–286]. Primary MSCs show heightened sensitivity to hypoxia, with both proliferation and differentiation being inhibited; Following passaging, the cells exhibited enhanced adaptability and stabilized osteogenic capacity [287]. Meanwhile, external strategies including culture medium composition, synergistic materials, and intervention modalities (intermittent hypoxia typically outperforms sustained hypoxia) can synergistically modulate oxygen concentration thresholds and optimize osteogenic outcomes [287,288]. For instance, an exposure frequency of twice to four times weekly over an intervention period of 5–8 weeks has been demonstrated to yield superior osteogenic effects. Excessively high frequencies or prolonged/abbreviated durations may compromise efficacy due to diminished cellular adaptability or insufficient signaling pathway activity [288–290]. However, the greatest challenge for *in vivo* applications is that tissue oxygen tension is not constant but rather a spatiotemporal dynamic variable influenced by factors such as blood flow and metabolism, making it difficult for any single approach to achieve optimal efficacy [291]. Therefore, the breakthrough direction for future research lies in transitioning from static interventions to dynamic precision control. By integrating real-time *in vivo* oxygen monitoring, multi-parameter dynamic surveillance, and the development of smart-responsive materials, we can achieve spatiotemporally precise delivery of oxygen tension, thereby truly advancing the clinical translation of MSC osteogenic differentiation strategies (Fig. 4).

Synergistic Strategies, Clinical Translation, and Future Directions

In the field of bone tissue engineering, utilizing physical stimulation to regulate the osteogenic differentiation of MSCs has demonstrated tremendous potential. However, the efficacy of single physical stimulation is often limited and fails to fully replicate the complex mechanical and biological environment *in vivo*. Consequently, current research paradigms have shifted from single-stimulus approaches to multi-signal synergy, from static environments to dynamic responses, and from *in vitro* simulation toward clinical translation (Fig. 5).

Multi-Signal Synergistic Enhancement Strategies

To maximize the induction of osteogenic differentiation in MSCs, researchers are actively exploring multi-signal enhancement strategies from two dimensions: “physical-biochemical synergies” and “physical-physical synergies”, aiming to precisely simulate the complex bone regeneration microenvironment *in vivo*. At the physical-biochemical synergy level, the core principle involves utilizing physical stimulation to enhance cellular responsiveness to biochemical factors, thereby achieving superior therapeutic efficacy with lower doses and mitigating side effects. For instance, FSS activates the YAP/TAZ mechanosignaling hub through integrin activation, significantly enhancing the osteogenic induction efficiency of BMP-2 [292,293]; Combining hypoxic preconditioning with three-dimensional culture synchronously enhances the osteogenic potential, survival rate, and homing ability of MSCs [294]. In physical-physical synergy, researchers are dedicated to constructing more realistic mechanical microenvironments, such as utilizing nanostructured scaffolds for sustained release of Zn^{2+} ions while applying FSS to synergistically promote osteogenesis and anti-infection [295]. Particularly noteworthy is the signal convergence and amplification effect among different mechanical signaling pathways. Research reveals that signals from FSS and HP ultimately converge on nuclear YAP1 and NFAT2. Their transcriptional complex formation more effectively initiates osteogenic gene expression than any single signal [119]. Additionally, thermo-mechanical coupling stimulation accelerates osteogenic differentiation by modulating calcium influx and histone modifications [296]. In conclusion, the synergistic strategy integrating multiple physical and biochemical signals has advanced from single-pathway studies to a new stage of multi-signal network integration. By activating key signaling nodes, simulating physiological microenvironments *in vivo*, and achieving signal convergence and amplification, this approach significantly enhances the osteogenic differentiation efficiency of MSCs. This progress lays a solid theoretical foundation for developing next-generation bone tissue engineering products and precise regenerative therapies.

Intelligent and Dynamic Biomaterial Systems

The deep integration of physical stimulation with intelligent dynamic biomaterials is pioneering new paradigms for osteogenic differentiation of MSCs through synergistic amplification effects. The core of this strategy lies in

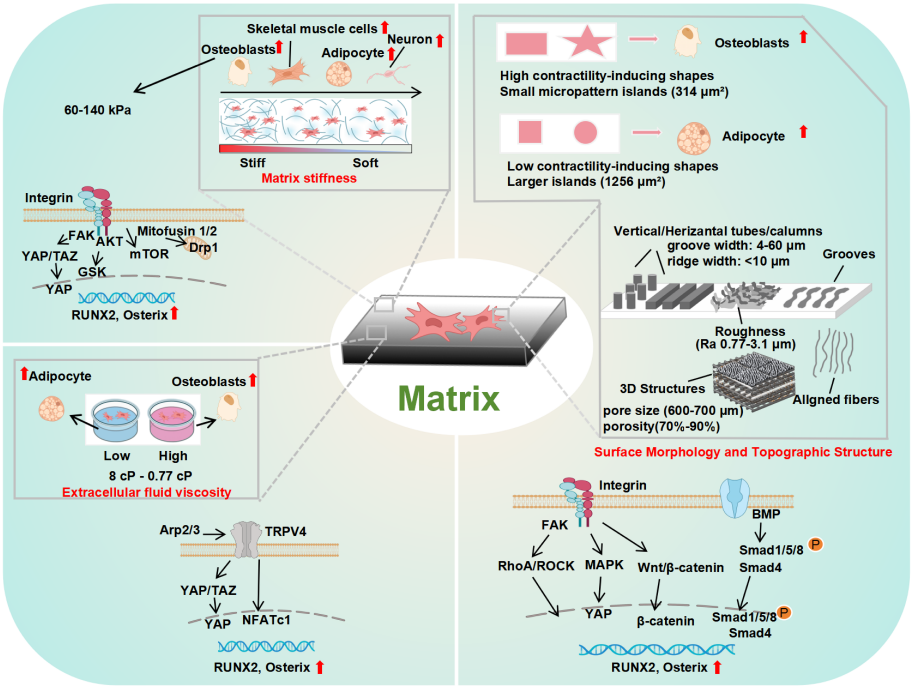

Fig. 4. Other physical microenvironmental factors affecting MSC osteogenic differentiation. Physical microenvironmental cues, including cell shape, substrate geometry, surface roughness, groove and fiber alignment, and three-dimensional pore structure, regulate MSC osteogenic differentiation by affecting cell adhesion, cytoskeletal organization, and downstream signaling pathways, ultimately activating osteogenic transcription factors such as RUNX2 and Osterix [12,72,204–206,220–225,232–236,242,257,258,261–265,284,289,290]. Created in BioRender. Zhang, F. (2026) https://BioRender.com/26qohnr.

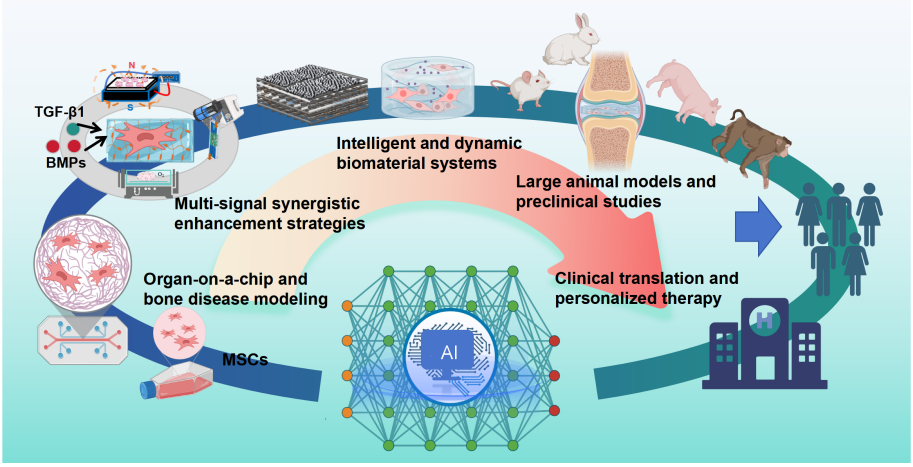

Fig. 5. Synergistic Physical Stimulation for MSC Osteogenic Differentiation. Future directions in physical stimulation-mediated regulation of MSC osteogenic differentiation include multi-signal synergistic enhancement strategies, intelligent and dynamic biomaterial systems, organ-on-a-chip and bone disease modeling, large animal models and preclinical studies, and clinical translation toward personalized therapy. The integration of biomaterials, microphysiological systems, and artificial intelligence-assisted optimization may improve the precision, efficacy, and translational potential of physical stimulation-based bone regeneration. MSCs, mesenchymal stem cells. Created in BioRender. Zhang, F. (2026) https://BioRender.com/e60fkbl.

utilizing smart materials such as piezoelectric and magnetically responsive materials as dynamic mediators to efficiently convert external physical signals—including magnetic fields, ultrasound, light, or mechanical forces—into mechanically, electrically, and chemically perceptible cues for cells. This process precisely activates key pathways such as MAPK/ERK and Wnt to promote osteogenic differentiation [189,297–299]. For instance, magnetically responsive materials undergo shape transformation and mechanical dynamics under magnetic fields, which can enhance cellular mechanosensitivity and subsequently activate osteogenic signaling pathways [298]. Electroactive substrate materials combined with electrical stimulation significantly enhance the osteogenic potential of MSCs while upregulating key osteogenic genes such as ALP and RUNX2 [300]. Building upon this foundation, smart materials integrated with physical stimulation can dynamically modulate the microenvironment, enabling spatiotemporally controlled release of growth factors and real-time modulation of matrix stiffness, thereby optimizing osteogenic conditions for MSCs [188,301]. For instance, near-infrared-activated 3D-printed hydrogel scaffolds enable controlled release of drugs and facilitate bone regeneration [301]. The stiffness modulation technique based on core-shell microfibers dynamically regulates the effective stiffness perceived by cells while preserving the biochemical properties of the matrix [302]. Utilizing 3D liquefied microcapsules to simulate the bone marrow microenvironment, or employing all-particulate bioprinting systems to address nutrient diffusion challenges, provides unprecedented ideal platforms for in-depth investigation of HP-mediated mechanotransduction mechanisms [117]. As a frontier extension in this field, 4D bioprinting technology significantly enhances *in vitro* simulation capabilities by constructing dynamically evolving structures across the temporal dimension. For instance, magnetically controlled 4D bioprinting that integrates diverse materials and drug delivery systems achieves synergistic interplay between morphological transformation and functional release under external magnetic fields, providing revolutionary solutions for complex bone defect repair [303,304].

Although smart dynamic biomaterials hold great potential for promoting the osteogenic differentiation of MSCs, their long-term clinical safety still faces numerous challenges. The primary safety challenges currently encountered by smart biomaterials in bone tissue engineering include insufficient long-term biocompatibility, the toxic risk of degradation products, abnormal immune responses, and uncertainties regarding the long-term *in vivo* behavior of special functional materials (e.g., iron ion accumulation, long-term effects of electrical stimulation parameters) [305]. According to the ISO 10993 standard system, implantable biomaterials must pass three tiers of toxicity testing: acute (short-term), subchronic (30 days), and chronic (over 12 months). However, most existing studies are limited to short-term observations (e.g., within 6 months), resulting in an insufficient systematic understanding of the long-term safety of various smart materials [306–308]. For instance, regarding iron-based materials, although the Fe/Zn composite developed by Tong *et al*. [306] demonstrated good mechanical properties and bioactivity during a 2-month implantation period, the accumulation of iron ions and the associated oxidative stress risk beyond 6 months post-implantation were not tracked. In the case of electroactive materials (e.g., PU/CNT scaffolds), the PDA@BTO thermoelectric nanocomposite film developed by Guo *et al*. [308] achieved excellent osteo-immunomodulatory effects through electrothermal synergy, but its *in vivo* study was limited to a 12-week observation period, lacking long-term genotoxicity data (e.g., mutation rates and apoptosis). Furthermore, although some 3D/4D printed PLGA scaffolds exhibit excellent initial mechanical properties, the long-term matching between their mechanical degradation rate and the rate of osteogenesis during *in vivo* and *in vitro* degradation has not been validated. These research gaps highlight the urgency of establishing a long-term biocompatibility evaluation system. Therefore, future research should prioritize the development of fully metabolizable dynamic materials (e.g., double-helical polymers) to reduce long-term retention risks, establish long-term implantation evaluation protocols for smart materials (including degradation product metabolism, functional stability, and genotoxicity), and conduct integrated analysis using multimodal monitoring techniques such as Micro-CT and blood biomarkers to achieve precise tracking of material behavior *in vivo*. Additionally, small-scale clinical trials should be conducted for the most promising materials (e.g., PLGA/BP scaffolds) to verify their clinical safety, thereby laying the foundation for the widespread application of smart biomaterials in bone tissue engineering.

Organ-on-a-chip and Bone Disease Modeling

Bone organ-on-a-chip platforms successfully recapitulate cellular interactions, three-dimensional matrices, and dynamic biomechanical-biochemical stimuli through highly biomimetic bone microenvironments engineered on microfluidic platforms, substantially improving physiological relevance and clinical predictability for various bone disease models including osteoporosis and osteoarthritis [309–311]. To precisely simulate the complex mechanical environment *in vivo*, bone-on-a-chip platforms integrate modules such as mechanical stretching, hydrodynamics, and EMFs [305,312,313]. These systems combine real-time imaging with electrochemical and optical biosensor technologies to continuously track cell differentiation and mineralization processes [309]. Subsequently, machine learning algorithms are employed to conduct deep analysis of massive datasets, providing scientific foundations for drug screening and personalized therapies [314]. Although challenges persist in accurately simulating bone

tissue complexity, achieving long-term culture, and facilitating clinical translation [313,315], researchers are actively addressing these through the development of bioactive materials, integration of AI-driven intelligent monitoring, and application of advanced manufacturing techniques [310,316,317]. Looking ahead, with continuous advancements in materials science, micro-nano fabrication technology, and biosensing technology, coupled with interdisciplinary innovation, bone organ chips will be able to construct highly personalized disease models with greater fidelity. They will precisely predict patient treatment responses and ultimately hold the potential to become disruptive tools for advancing precision medicine in bone diseases and new drug development, ushering orthopedics into a new patient-centered era.

Large Animal Models and Preclinical Studies

Studying the regulation of physical stimulation on osteogenic differentiation of MSCs in large animal models constitutes a highly complex systematic project. Its success relies on comprehensive optimization of cell sources, pretreatment methods, physical stimulation parameters, animal model selection, and multidimensional evaluation systems [318,319]. First, the source and *in vitro* pretreatment of MSCs are prerequisites for determining osteogenic efficacy. Cells from different sources exhibit variations in differentiation potential, while pretreatment methods such as electrical stimulation, hypoxia, or heat shock can optimize osteogenic effectiveness by enhancing differentiation capacity, regulating differentiation balance, or improving immunomodulatory functions, respectively [285,320]. Second, precise control of physical stimulation parameters is crucial for regulating cellular behavior, requiring optimization of frequency, strength, and duration [204,321]. Ideal models should simulate clinically common traumatic fractures or site-specific defects (e.g., segmental defects in rabbit femurs or porcine acetabular defects), with precise design of critical size and mechanical stability to authentically reflect clinical challenges [322,323]. Furthermore, to comprehensively evaluate osteogenic outcomes, a multidimensional evaluation system encompassing imaging, histology, biomechanics, and molecular biology must be established. This system should enable holistic analysis of bone formation volume, tissue morphology, functional recovery, and molecular mechanisms [324–327]. Despite the promising prospects, this field still faces challenges including the absence of parameter standardization, the complex *in vivo* behavior of MSCs, limited stimulation modalities, and clinical translation bottlenecks. Future research should focus on standardizing stimulation parameters, elucidating MSC heterogeneity and immunomodulatory mechanisms, and advancing integrated innovation combining multimodal physical stimulation with advanced biomaterials.

Clinical Translation Challenges and Personalized Therapy

The clinical translation of MSCs for osteogenic differentiation faces interconnected systemic challenges. Macroscopically, ethical controversies and globally inconsistent regulatory policies constitute high entry barriers. The diverse sources of MSCs introduce complex ethical considerations, and while their manufacturing processes require GMP compliance, safety concerns such as immune rejection and tumorigenic risks lack clear regulatory frameworks. Even promising cell-free therapies require further refinement of quality control standards [328,329]. At the mesoscale level, physical stimulation interventions suffer from the absence of a standardized experimental parameter system, resulting in challenges in efficacy evaluation and reproducibility. Substantial discrepancies in stimulation parameters across studies and their complex interactions with microenvironmental factors significantly impede research integration [330]. At the microscale level, the regulatory mechanisms governing MSC osteogenic differentiation—including discrepancies between *in vitro* and *in vivo* settings, senescent states, and immune microenvironment influences—have not been fully elucidated. This knowledge gap fundamentally undermines the reliability and reproducibility of foundational research [331,332].

In addition, the safety of physical stimulation itself and its potential side effects are key risks that cannot be ignored in the clinical translation process. For example, Huang *et al*. [333] demonstrated that excessive mechanical compression can cause various damages to MSCs, including reduced cell viability, induced apoptosis, mitochondrial dysfunction, oxidative stress, and destruction of cellular ultrastructure, etc. Similarly, Liu *et al*. [334] revealed that mechanical stimulation has a “double-edged sword” effect on MSCs, with a clear “safety boundary”. While moderate mechanical stimulation can reverse MSC aging, high-frequency or large-amplitude mechanical stretching constitutes excessive mechanical stimulation, which can induce strong DNA damage in cells, thereby exacerbating the aging process and inflammatory response. Therefore, the main bottleneck currently faced in this field is the lack of quantitative safety assessment standards, making it difficult to accurately define the boundaries of the “therapeutic window”, which increases the uncertainty of clinical applications.

To overcome these bottlenecks, the central strategy for future development lies in achieving precise personalized treatment. Firstly, precise matching should be conducted based on differences in proliferation capacity, differentiation potential, and immunomodulatory characteristics among MSCs from various sources, tailored to individual patient conditions and therapeutic needs [335–338]. Secondly, it is essential to revolutionize the application of physical stimulation by moving beyond traditional fixed-parameter approaches and introducing closed-loop control systems. Through real-time monitoring of patient phys-

iological signals and cellular responses, considering dynamic changes in patient physiological status and cellular reaction characteristics during treatment, artificial intelligence algorithms should be employed to dynamically adjust stimulation parameters for optimal stimulation strength and rhythm. This strategy has achieved success in platforms like EcoAI for treating chronic pain and can be adapted to the field of MSC osteogenic differentiation [339,340].

Conclusions

In summary, physical stimulation demonstrates tremendous clinical application potential as a non-invasive, highly controllable, and versatile regulatory strategy for promoting MSC osteogenic differentiation and accelerating bone tissue regeneration. Its underlying mechanisms span multiple dimensions—from cellular signaling pathway activation and mechanical microenvironment perception to metabolic reprogramming and enhanced extracellular vesicle functionality—forming a sophisticated regulatory network. Looking ahead, the development in this field will focus on three core directions: first, achieving precise optimization of stimulation parameters and constructing multi-modal synergistic therapeutic strategies through integration of novel functional materials with intelligent responsive systems; second, leveraging interdisciplinary integration to uncover more refined molecular mechanisms; third, accelerating rigorous clinical validation and translational applications. Through breakthroughs in these critical areas, physical stimulation technologies are poised to lead qualitative leaps in bone regeneration therapy. This advancement will not only provide revolutionary solutions for addressing bone defect repair and skeletal diseases, but also establish new paradigms for the entire field of regenerative medicine.

List of Abbreviations

2D, two-dimensional; 3D, three-dimensional; ALP, alkaline phosphatase; AR, aspect ratio; ASCs, adipose-derived MSCs; BMP, bone morphogenetic protein; BMSCs, bone marrow-derived MSCs; DC, duty cycle; ECM, extracellular matrix; EMFs, electromagnetic fields; ES, electrical stimulation; EVs, extracellular vesicles; FSS, fluid shear stress; GelMA, gelatin methacrylamide; HP, hydrostatic pressure; ITGA11, integrin α11; LEDs, light-emitting diodes; LIPUS, low-intensity pulsed ultrasound; MSCs, mesenchymal stem cells; OCN, osteocalcin; OPN, osteopontin; PAAm, polyacrylamide; PBM, photobiomodulation; PEG, polyethylene glycol; PEMFs, Pulsed electromagnetic fields; ROS, reactive oxygen species.

Availability of Data and Materials

Not applicable.

Author Contributions

FFZ and LW contributed to the conception and design of this review and drafted the manuscript. ML, YLL, and MYZ contributed to the literature collection and analysis. JTL contributed to the critical revision of the manuscript for important intellectual content. All authors contributed to the interpretation of the literature, read and approved the final manuscript, and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics Approval and Consent to Participate

Not applicable, as this study is a review article and does not involve human participants or animal subjects.

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Conflict of Interest

The authors declare no conflict of interest.

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