

Mechanical-immune feedback regulation in distraction osteogenesis: From mechanotransduction to immune microenvironment remodeling (Review)

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Abstract. Distraction osteogenesis (DO) is a well-established regenerative technique in which controlled tensile forces stimulate *de novo* bone formation between gradually separated bone segments. Although traditionally interpreted as a mechanically driven process, accumulating evidence indicates that successful DO depends on the coordinated regulation of mechanotransduction, immune microenvironment remodeling, angiogenesis, osteogenesis and subsequent bone remodeling. The present review summarizes current advances in the biological mechanisms of DO and proposes an integrative mechanical-immune feedback framework to explain how mechanical cues and immune responses interact throughout the regenerative process. During the latency phase, osteotomy-induced sterile injury initiates hematoma formation, inflammatory cell recruitment and early cytokine release, thereby establishing a preparatory microenvironment for tissue repair. During the distraction phase, various mechanical parameters, such as rate, rhythm, frequency and local tissue stiffness, are sensed by mechanosensitive structures, including integrin-focal adhesion complexes, primary cilia, mechanosensitive ion channels and cytoskeleton-associated nuclear signaling systems. These signals in turn activate downstream signaling pathways, including MAPK, Wnt/ β -catenin, bone morphogenetic protein-Smad, Akt/mTOR/NF- κ B and Ras homolog family

member A/Yes-associated protein/transcriptional co-activator with PDZ-binding motif, which collectively promote osteogenic differentiation, matrix deposition and vascular remodeling. In parallel, mechanical stimulation can reshape the local immune milieu by regulating macrophage polarization, cytokine balance, osteoclast-osteoblast coupling and angiogenesis-osteogenesis interactions. During consolidation, immune-mediated remodeling, extracellular matrix maturation and coordinated bone formation and resorption contribute to mineralization and structural integration of the regenerate. Based on these phase-specific events, DO can be conceptualized as a dynamic process involving mechanical input, mechanotransduction, immune modulation, vascularized osteogenesis and remodeling feedback. This mechanical-immune perspective provides a structured basis for understanding heterogeneity in DO outcomes across anatomical sites, age groups and pathological conditions, which may inform future optimization of distraction protocols, biomarker-guided monitoring and targeted immunomodulatory strategies.

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1. Introduction

Distraction osteogenesis (DO) is a well-established regenerative medicinal technique that harnesses controlled mechanical traction to induce *de novo* bone formation, with broad clinical

applications in the treatment of bone defects and limb lengthening (1,2). First developed by Soviet surgeon Gavriil A. Ilizarov (3,4) in the mid-20th century, DO is grounded in the ‘law of tension-stress’, which posits that gradual and sustained mechanical strain can activate intrinsic regenerative capacity in skeletal tissues, thereby promoting bone formation and maturation (3,4). Despite its clinical efficacy, the therapeutic process is prolonged and biologically intricate, involving not only mechanobiological responses of bone-forming cells but also coordinated inflammatory and immune regulatory events (5). In recent years, the emerging field of osteoimmunology has highlighted the bidirectional crosstalk between immune and skeletal systems, underscoring that immune cells can actively modulate bone remodeling through cytokine signaling, cell-cell interactions and microenvironmental reprogramming (6-8). Within the context of DO, a unique model of mechanically driven regeneration, the interplay between mechanical cues and the evolving immune microenvironment has become a focal point for understanding the systemic nature of tissue regeneration.

To address this complexity, a mechanical-immune closed-loop regeneration framework has been proposed, with a conceptual regulatory framework in regenerative medicine that conceptualizes ‘regeneration’ as a dynamic, feedback-regulated process. Specifically, mechanical stimuli are transduced into biochemical signals through a number of mechanosensitive pathways, such as integrin-mediated adhesion, primary cilia sensing and ion channel activation, thereby influencing cellular behavior and fate decisions (9,10). Concurrently, these mechanical inputs can reshape the local immune landscape, particularly through modulation of macrophage polarization and cytokine profiles (11,12). In turn, immune-derived mediators feed back to regulate osteogenesis, angiogenesis and extracellular matrix remodeling, forming a self-reinforcing regenerative circuit. While prior studies have largely examined either the biomechanical or immunological aspects of DO in isolation, there remains a critical gap in the systematic integration of these two dimensions (5,13,14).

Therefore, to address this gap, the present review amalgamate current evidence regarding the spatiotemporal evolution of tissues, mechanosensitive signaling pathways, immune microenvironment remodeling and angiogenesis-osteogenesis coupling during DO. The present review summarizes current evidence and proposes a mechanical-immune feedback framework for understanding distraction osteogenesis. Relevant literature was searched in PubMed (<https://pubmed.ncbi.nlm.nih.gov/>), Web of Science (<https://www.webofknowledge.com/>) and Google Scholar (<https://scholar.google.com/>) using combinations of the following terms: ‘distraction osteogenesis’, ‘mechanotransduction’, ‘mechanobiology’, ‘osteoimmunology’, ‘immune microenvironment’, ‘macrophage polarization’, ‘M1 macrophage’, ‘M2 macrophage’, ‘angiogenesis-osteogenesis coupling’, ‘osteoclast-osteoblast coupling’, ‘integrin-focal adhesion kinase (FAK)’, ‘primary cilia’, ‘Piezo type mechanosensitive ion channel component 1 (Piezo1)’, ‘Yes-associated protein (YAP)/transcriptional co-activator with PDZ-binding motif (TAZ)’, ‘Ras homolog family member A (RhoA)/Rho-associated protein kinase (ROCK)’, ‘Wnt/ β -catenin’, ‘bone morphogenetic protein (BMP)-small mothers against decapentaplegic (Smad)’,

‘mammalian target of rapamycin (mTOR)’, ‘nuclear factor κ -light-chain-enhancer of activated B cells (NF- κ B)’, ‘vascular endothelial growth factor (VEGF)’ and ‘type H vessels’. Priority was given to experimental, translational and clinical studies directly investigating DO models, including mandibular, craniofacial, sutural and long-bone DO. When direct DO-specific evidence was limited, closely associated studies on fracture healing, cranial suture expansion, skeletal mechanobiology, bone defect repair and pathological bone-regeneration models were considered to support mechanistic interpretation. By integrating these findings, the present review proposes a mechanical-immune closed-loop regulatory framework in which mechanical cues are transduced into osteogenic and angiogenic signals whilst concurrently reshaping the immune milieu, where immune-derived mediators in turn influence the efficiency, direction and remodeling trajectory of mechanically induced bone regeneration. Within this framework, the present review further discusses conserved biological principles and context-dependent heterogeneities across anatomical sites, age groups and pathological conditions, whilst outlining potential translational strategies, including optimization of distraction protocols, biomarker-guided monitoring and targeted immunomodulatory interventions.

2. Stage-specific mechanical encoding and tissue fate in DO

DO proceeds through three consecutive and functionally distinct phases, each defined by a stage-specific mechanical microenvironment that ‘encodes’ a unique tissue regeneration trajectory (5,15). The first phase, known as the latency period or delay phase, spans the interval between osteotomy and the initiation of distraction, typically lasting 5-7 days. During this phase, the surgical site undergoes early inflammatory repair, characterized by hematoma formation, infiltration of inflammatory cells and initial recruitment of chondroprogenitor cells and fibrous tissue precursors (16).

The second phase, known as the distraction phase, is initiated by applying incremental mechanical traction, typically at rates of 0.25-1.0 mm per 12 h, which progressively separates the osteotomized bone segments. Within the interfragmentary gap, also termed the fibrovascular zone, tensile stress induces the development of a spatially organized regenerative structure. Centrally, a highly vascularized fibrous tissue rich in fibroblasts and nascent capillaries forms the so-called ‘distraction zone’, also known as fibrous callus, whereas regions adjacent to the bone stumps exhibit islands of cartilage and early woven bone deposition (15,16). Previous animal studies have demonstrated that slow and sustained tensile strain can promote direct intramembranous ossification, whereas excessive or accelerated distraction predisposes the bone to aberrant chondrogenesis and compromised bone quality (16,17). This phenomenon underpins the concept of ‘mechanical encoding’, in which specific mechanical parameters, such as distraction rate, rhythm and frequency, transmit instructive cues to resident mesenchymal and stromal cells, thereby guiding their proliferative activity and lineage commitment (18,19). In mandibular DO among elderly patients, a slower distraction rate (0.5 mm/day) associates with enhanced vascular perfusion and robust osteogenic activity, whereas a higher

rate (1.0 mm/day) is associated with diminished vascularity and delayed bone formation (18). These findings underscore that the mechanical milieu can actively programme regenerative outcomes through modulation of local angiogenesis and cellular mechanostasis.

In the late distraction phase, central fibrous tissues begin to mineralize and evolve into primary trabecular bone, whilst transitional cartilaginous regions undergo endochondral ossification (15). The third and final phase, known as the consolidation, healing or mineralization phase, begins upon cessation of distraction. Although mechanical loading is no longer actively applied, the external fixation device must be maintained for several weeks to months to ensure structural integrity. In this quiescent biomechanical environment, newly formed bone undergoes progressive mineralization, maturation and integration with the host skeletal architecture (20,21). Histologically, this phase is marked by the remodeling of immature woven bone into structurally organized lamellar bone, reduction in callus volume and a significant increase in bone mineral density. Premature removal of fixation or inadequate consolidation duration may result in delayed bone union or fracture of the regenerate (2,22).

Tissue regeneration during DO follows a well-defined spatiotemporal sequence. In the initial phase, tensile stress acts on cells and the extracellular matrix, inducing morphological changes and activating key molecular signaling pathways. Subsequently, vascular endothelial cells and fibroblasts migrate and proliferate to establish a regenerative scaffold. Finally, osteoblasts differentiate and mediate matrix mineralization (17,18). This cascade of biological events is dynamically modulated by the post-traumatic immune-inflammatory response (Fig. 1). Temporally, the latency phase of DO corresponds to the inflammatory phase of conventional fracture healing, whilst the distraction phase aligns with the reparative phase. However, due to the sustained application of mechanical stimulation, the local microenvironment in DO markedly differs from that of natural healing. Distraction-induced tensile stress sustains elevated levels of osteogenic signaling, suppresses excessive chondrogenic maturation and promotes direct bone formation predominantly through intramembranous ossification (23,24). Sato *et al* (15) previously demonstrated in a rat femoral DO model that substantial cartilaginous tissue formed centrally within the callus in the absence of distraction, whereas under standard distraction conditions, the central region consisted primarily of fibrous tissue undergoing direct ossification, with cartilage largely confined to the peripheral regions. This mechanically driven inhibition of chondrogenesis is regarded as a pivotal mechanism underlying the efficient intramembranous bone formation observed in DO.

Immune cells serve a critical role in tissue remodeling throughout all stages of DO. During the latency phase, bone injury elicits an acute inflammatory response characterized by the rapid infiltration of neutrophils and macrophages, which clear necrotic debris and release a spectrum of cytokines (16,22,23). Evidence indicates that in early mandibular DO in rabbits, systemic IL-6 concentrations rise shortly after surgery, whilst insulin-like growth factor (IGF)-1 levels decline (16). Upon initiation of distraction, these systemic markers gradually return to baseline, reflecting a shift from

inflammation toward active tissue regeneration. Throughout the distraction phase, persistent mechanical loading establishes a distinct local immune milieu. Moderate tensile strain helps restrain excessive inflammation whilst simultaneously enhancing angiogenesis and osteogenic activity (17,18). Experimental findings further reveal that TNF- α expression in newly formed bone tissue does not significantly increase during DO, since TNF receptor knockout mice are still capable of completing bone elongation (16,25). These results imply that in the context of intramembranous ossification-dominated DO, the roles of certain canonical proinflammatory mediators, such as TNF- α , may be attenuated or functionally compensated by mechanical cues. Collectively, stage-specific mechanical cues in DO precisely orchestrate cellular fate decisions and differentiation trajectories, driving a structured progression from inflammatory infiltration to fibrous tissue formation and ultimately to *de novo* osteogenesis. Concurrently, the immune system functions as a continuous regulatory framework, supplying essential initiating signals and dynamic modulation, thereby forming a highly integrated and coordinated regenerative feedback system (Fig. 1).

3. Mechanotransduction axis

Mechanotransduction refers to the biological process by which cells convert external mechanical stimuli into intracellular biochemical signals. In DO, the mechanotransduction axis constitutes the central mechanism through which osteoblasts and their surrounding microenvironment respond to tensile forces (26). Key mechanical parameters include traction amplitude (displacement), frequency (typically twice daily), rate (0.5 mm/12 h) and total duration (18). Variations in these parameters result in distinct mechanical environments on the cellular level. Continuous traction generates sustained tensile stress, whereas intermittent traction establishes a periodic stress-relaxation cycle. A slower distraction rate facilitates tissue adaptation and neogenesis, while excessively rapid distraction may induce tissue injury and maladaptive stress responses (17,18). Therefore, optimization of mechanical parameters is critical for improving osteogenic efficiency and the structural quality of regenerated bone, representing a pivotal direction in refining clinical DO protocols. In a previous *in vitro* study simulating uniaxial cyclic tensile strain within the DO gap, Bhatt *et al* (27) demonstrated that the expression of osteogenic markers BMP-2 and type I collagen peaked at a 9% strain magnitude, with diminished responses observed at both higher and lower strain levels, indicating the existence of an optimal mechanical stimulation window. Furthermore, adjunctive physical modalities, including low-intensity pulsed ultrasound (LIPUS), have been applied to enhance bone maturation and mineralization during DO (28).

Cells utilize multiple mechanosensors to detect and transduce mechanical cues. Within the bone tissue, canonical mechanoresponsive cells include lineage-specific osteoblasts, osteocytes and mesenchymal stem cells (MSCs). Osteocytes, embedded within the mineralized matrix and interconnected through an extensive dendritic network, are particularly sensitive to matrix deformation and fluid shear stress. Upon mechanical loading, they rapidly release key

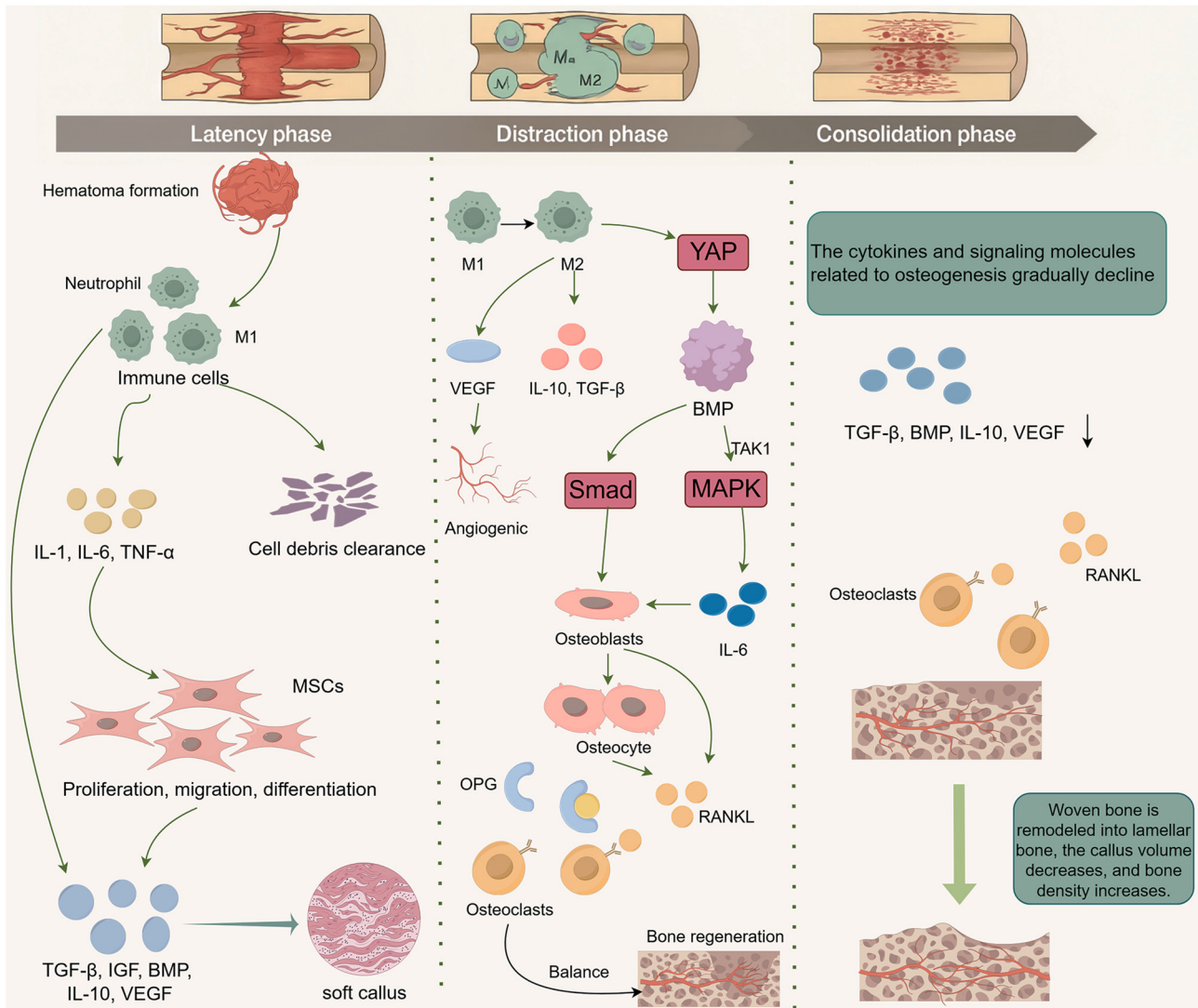


Figure 1. Immune-related processes during distraction osteogenesis. During the latency phase, osteotomy-induced hematoma formation and sterile inflammation promote neutrophil and macrophage recruitment, debris clearance, cytokine release and early activation of reparative cells. M1-polarized macrophages and inflammatory mediators contribute to the initiation of tissue repair, whilst MSCs and osteoprogenitor cells begin to migrate, proliferate and undergo osteogenic priming. During the distraction phase, controlled tensile loading promotes mechanotransduction and progressively reshapes the immune microenvironment. A timely transition from M1-dominant inflammation toward M2-associated repair supports angiogenesis, osteoblast differentiation, extracellular matrix deposition and vascularized bone formation through mediators, such as IL-10, TGF- β , VEGF, BMP-related signals and IL-6. During the consolidation phase, inflammatory activity gradually resolves, while osteoblast-osteoclast coupling, RANKL/RANK/OPG signaling, matrix mineralization and lamellar remodeling contribute to structural maturation and integration of the regenerate. MSCs, mesenchymal stem cells; IGF, insulin-like growth factor; TAK1, TGF β -activated kinase 1; BMP, bone morphogenetic protein; OPG, osteoprotegerin; RANKL, Rank ligand; YAP, Yes-associated protein.

signaling molecules, including prostaglandin E2, adenosine triphosphate (ATP) and nitric oxide (NO), to initiate downstream signaling cascades (10). Under sustained tensile stress, MSCs exhibit a strong propensity toward osteogenic differentiation, accompanied by the significant upregulation of core transcription factors, such as bone γ -carboxyglutamate protein, RUNX family transcription factor 2 (Runx2) and BMP2 (29). Emerging evidence further identified subsets of bone marrow stromal progenitor cells with heightened sensitivity to mechanical stretch. GLI family zinc finger 1 (Gli1)⁺ suture-derived stem cells have been found to be specifically activated during cranial suture DO, showing upregulation of osteogenic differentiation-related genes and activation of primary cilia-mediated Hedgehog signaling compared with non-distraction control sutural cells, suggesting their

potential role as a major cellular source for *de novo* bone formation (30,31). At the molecular level, the integrin-focal adhesion complex serves as a primary mechanosensory structure. Mechanical stretching of the cytoskeleton leads to the phosphorylation and activation of FAK at integrin-mediated focal adhesion sites, thereby converting mechanical forces into intracellular biochemical signals (32,33). Tong *et al* (33) reported robust FAK expression during distraction in a rat mandibular DO model, with bone sialoprotein mRNA localization patterns mirroring FAK immunolocalization; related mandibular DO evidence has also shown expression of BMP-2 and BMP-4. By contrast, FAK levels were minimal in non-distraction control defects. These findings strongly support the essential role of the integrin/FAK signaling pathway in mediating distraction-induced osteogenesis.

Another critical mechanotransduction mechanism involves the physical linkage between the cytoskeleton and the nuclear lamina. Under tensile stress, increased tension within the actin-myosin cytoskeletal network is transmitted to the nucleus through linker proteins, such as Nesprin-Sad1 and UNC-84 domain-containing protein complexes, enabling direct regulation of gene expression programs (34,35). YAP and TAZ, core effectors of the Hippo signaling pathway, are well-established mechanoresponsive transcriptional co-activators. In DO-related mechanical models, YAP/TAZ undergo pronounced subcellular relocalization, shifting from cytoplasmic retention to nuclear accumulation, thereby activating the transcription of osteogenesis-related genes, including Runx2, Osterix and alkaline phosphatase (ALP) (36-38). Previous evidence indicates that tensile stress can activate the RhoA/ROCK signaling axis, stabilizing actin stress fibers and promoting YAP/TAZ nuclear translocation, which enhances the osteogenic commitment of mesenchymal cells (36,39). Conversely, inhibition of RhoA or genetic silencing of YAP/TAZ markedly attenuated mechanically induced osteogenesis, underscoring the functional necessity of this pathway in force-driven bone formation (14,36). Additionally, the mechanosensitive ion channel Piezo1 serves a significant role in osteocyte mechanosensing (40). Although its precise role in DO remains under investigation, currently available data suggest that mechanical loading activates Piezo1, triggering Ca^{2+} influx, which in turn promotes calcium-dependent TAZ nuclear translocation, modulates mitochondrial dynamics, stimulates ATP release and activates downstream pathways, such as MAPK/ERK (40,41). These processes collectively regulate cell proliferation, differentiation and tissue regeneration, positioning Piezo1 as a potential synergistic mediator in the mechanobiological response during DO (41).

Mechanotransduction signaling network. The mechanotransduction signaling network is highly complex, yet its core response mechanisms can be categorized into four functional modules in total.

Rapid ion and small-molecule signaling. During the early phase of tensile stimulation, osteocytes rapidly release ATP, activating P2 receptors or induce Ca^{2+} influx through mechanically gated calcium channels, thereby initiating downstream signaling cascades (10,41). Additionally, NO and cyclic adenosine monophosphate levels rise significantly within minutes of mechanical loading, classifying them as primary mechanoresponsive messenger molecules (10).

Kinase signaling cascades. Mechanical forces are transduced through integrin-mediated activation of FAK, which subsequently activates multiple downstream kinase pathways, including the MAPK (ERK1/2, p38) and PI3K/Akt pathways (42,43). Of particular significance are mTOR and NF- κ B, two key downstream effectors of Akt. Wang *et al* (29) demonstrated that cyclic tensile strain can induce phosphorylation and activation of Akt in osteoblasts, concomitant with the coordinated activation of mTOR and NF- κ B. Inhibition of mTOR reduces NF- κ B activity and *vice versa*, indicating a positive feedback loop that synergistically enhances the expression of osteogenic genes. This illustrates a paradigm of functional integration in mechanotransduction through cross-pathway crosstalk. The mTOR pathway promotes

osteogenic differentiation by enhancing protein synthesis and cellular metabolism whilst moderately activated NF- κ B, despite its classical association with inflammation, also contributes to osteogenic induction under mechanical stimulation (29). These findings provide critical insights into the interplay between mechanical cues and immunomodulatory processes during bone regeneration.

Transcriptional regulatory networks. Mechanical signals ultimately drive reprogramming of gene expression in the nucleus. Beyond YAP/TAZ, the aforementioned MAPK and Akt pathways activate key transcription factors, such as Runx2 and Osterix, master regulators of osteogenesis (29,44). Experimental evidence suggested that mechanical stretching can robustly upregulate the expression of various growth factors and bone matrix proteins, including BMP-2/4, type I collagen, osteopontin and alkaline phosphatase (15,29). *In vivo* studies by Sato *et al* (15) previously revealed that the mRNA expression levels of BMP-2 and BMP-4 in callus tissues increased rapidly upon the initiation of distraction, remain elevated throughout the distraction phase and gradually decline after consolidation began. This stimulus-responsive expression pattern underscores the precise spatiotemporal control exerted by mechanotransduction over the osteogenic molecular network. Similarly, *in vitro* models showed that 8 h tensile stress can significantly elevate TGF- β 1 and IGF-1 mRNA expression levels in osteoblasts, with additional upregulation of bone-derived fibroblast growth factor and IL-6 observed after 24 h (45). Notably, mechanical stimulation selectively modulates proinflammatory gene expression. Under identical conditions, IL-1 β levels remain unchanged. This indicates a preferential activation of pro-anabolic and osteogenic pathways without eliciting non-specific inflammatory responses.

Regulation of angiogenic signaling. Successful DO critically depends on concurrent angiogenesis, a process actively regulated by mechanotransduction. Tensile stress induces osteoblasts and MSCs to secrete pro-angiogenic factors, such as VEGF (46,47). An *in vitro* study previously reported a ≤ 32 -fold increase in VEGF expression in osteoblasts subjected to 9% strain compared with static controls (27). Clinical analyses further demonstrate a significant positive association between microvessel density and new bone formation in regenerated tissues (18). Morgan *et al* (48) proposed through computational modeling that tissue elongation must be accompanied by proportional neovascularization. Otherwise, inadequate perfusion would lead to failed regeneration. Therefore, the mechanotransduction pathway is intricately linked to the 'angiogenesis-osteogenesis coupling' process, primarily through mechanisms, such as hypoxia-inducible factor-1 α /VEGF signaling, ensuring sufficient vascular supply and metabolic support for *de novo* bone formation.

In summary, the mechanotransduction axis constitutes a comprehensive regulatory system that spans the sensing of physical mechanical cues, the activation of cell surface receptors and the downstream initiation of intracellular molecular signaling networks (Fig. 2). Given that tissue regeneration in DO is primarily driven by externally applied and controllable mechanical stimuli, DO serves as an ideal model for investigating the fundamental mechanisms of mechanobiology. A detailed analysis of key signaling nodes, such

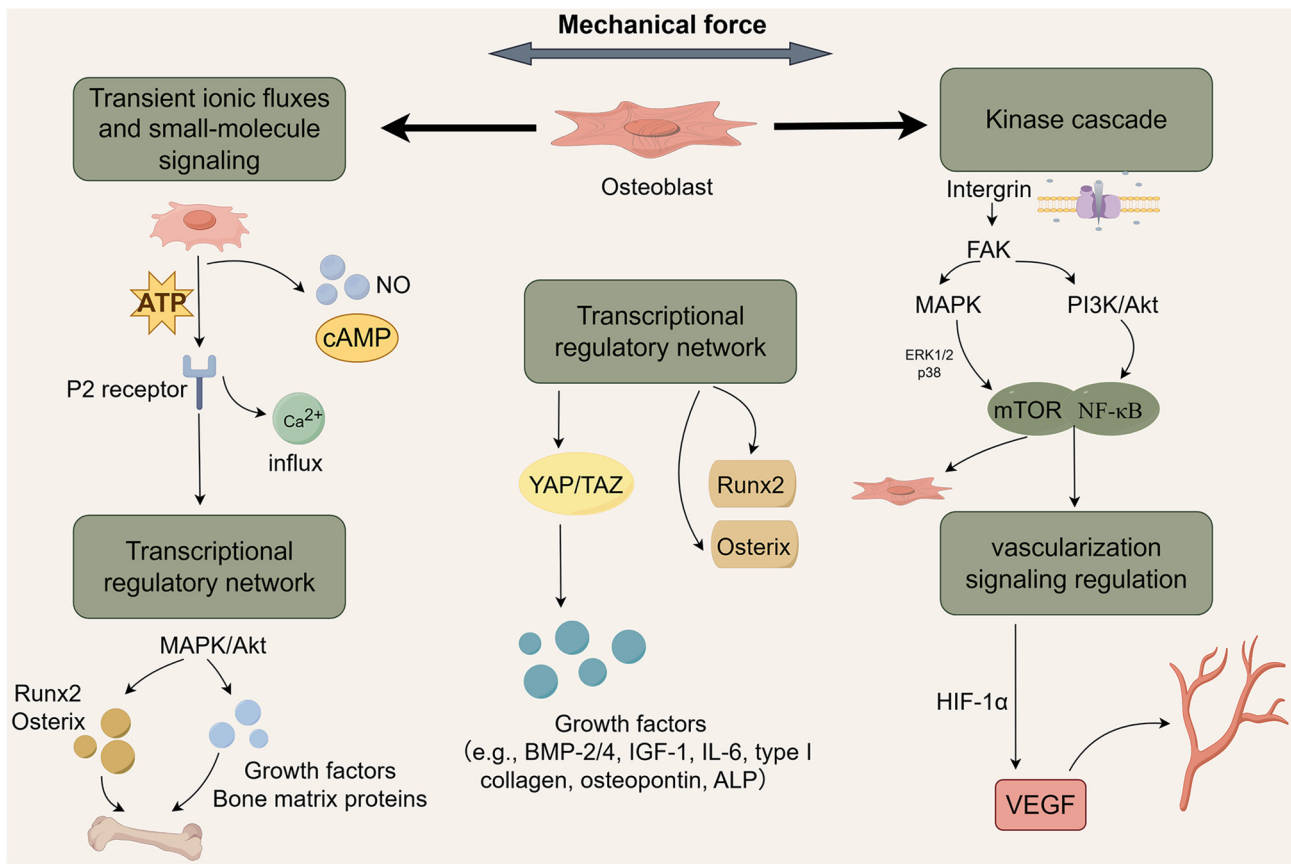


Figure 2. Core response mechanisms of the mechanotransduction signaling pathway. Mechanical tensile forces are sensed by multiple mechanosensitive structures, including integrin-focal adhesion complexes, primary cilia, mechanosensitive ion channels, cytoskeletal networks and cytoskeleton-associated nuclear signaling systems. Early responses include ATP release, Ca²⁺ influx, NO production, cAMP signaling and activation of focal adhesion kinase. These events further activate downstream pathways, such as MAPK, PI3K/Akt, Wnt/ β -catenin, BMP/Smad, Akt/mTOR/NF- κ B and RhoA/YAP/TAZ. Together, these pathways regulate Runx2, Osterix, ALP, type I collagen, osteopontin, BMPs and other osteogenic mediators. Mechanical stimulation also promotes angiogenesis-osteogenesis coupling through HIF-1 α /VEGF-related signaling, supporting endothelial cell activity, vascular remodeling and mineralized matrix formation within the distraction gap. HIF, hypoxia-inducible factor; BMP, bone morphogenetic protein; NO, nitric oxide; cAMP, cyclic adenosine monophosphate; IGF, insulin-like growth factor; ALP, alkaline phosphatase; YAP, Yes-associated protein; TAZ, transcriptional co-activator with PDZ-binding motif; FAK, focal adhesion kinase; Runx2, RUNX family transcription factor 2.

as integrin/FAK, RhoA/YAP and Akt/mTOR/NF- κ B, can facilitate the identification of potential therapeutic targets and advance the development of precision regulatory strategies (Table I). However, bone regeneration is not mediated solely by mechanical factors. It also requires coordinated interactions between the immune system and the local biochemical microenvironment. This interdependence underscores the existence of a second complementary regulatory axis, namely the immune crosstalk pathway, which operates in concert with mechanotransduction to orchestrate effective bone repair.

4. Osteoimmunology axis

During bone regeneration, the immune system exerts a dual-edged effect. Moderate inflammatory responses typically facilitate the initiation of tissue repair programs, whereas excessive or dysregulated inflammation may impair the healing process (11,49–51). In DO, the immune interaction axis involves the dynamic participation of a diverse range of immune cell types and the precise regulation of their secreted factors on osteogenic activity and bone remodeling. Macrophages serve as the central regulators of immune modulation, orchestrating

the regenerative microenvironment through phenotypic polarization shifts across distinct phases. In the early stages of DO, M1-polarized macrophages predominate and perform proinflammatory functions, including clearance of necrotic tissues and bone debris, in addition to the secretion of proinflammatory cytokines, such as TNF- α and IL-1 β (16). These actions promote increased vascular permeability and extracellular matrix remodeling. As the repair process advances, macrophages gradually transition towards the M2 phenotype, acquiring anti-inflammatory and pro-reparative properties. These M2 macrophages secrete mediators, such as IL-10 and TGF- β , which support osteoblast recruitment and differentiation whilst facilitating the resolution of inflammation (14,50,52,53) (Fig. 3). This M1-to-M2 polarization shift is widely recognized as a pivotal determinant of bone healing outcomes. Evidence indicates that promoting macrophage polarization toward the M2 phenotype significantly enhances bone repair efficiency, whereas sustained dominance of the M1 phenotype is strongly associated with impaired or delayed union (54). In the DO model, mechanical tensile stress may modulate macrophage polarization. Emerging studies have suggested that continuous mechanical stimulation can attenuate

Table I. Major mechanotransduction pathways involved in DO and related bone-regeneration models.

Pathway or mechanosensor	Main responsive cells	Main biological function	Evidence source	Role in DO	(Refs.)
Integrin-FAK signaling	Osteoblasts, MSCs, osteoprogenitor cells	Converts extracellular matrix deformation and cytoskeletal tension into intracellular kinase signaling; promotes osteogenic differentiation and matrix deposition	DO-specific animal and <i>in vitro</i> mechanical loading studies	Strong evidence for involvement in distraction-induced osteogenesis	(32,33,118)
Primary cilia-Hedgehog signaling	Suture-derived stem cells, osteoprogenitor cells, MSCs	Senses mechanical stimulation and regulates osteogenic lineage commitment, particularly in craniofacial and sutural tissues	Craniofacial and sutural DO-related studies	Important in craniofacial/sutural DO; broader relevance requires further validation	(30,31,99)
Piezo1-Ca ²⁺ signaling	Osteocytes, MSCs, macrophages	Mediates mechanically induced Ca ²⁺ influx, ATP release, mitochondrial adaptation, and downstream osteogenic or immunomodulatory signaling	Bone mechanobiology and emerging DO-related evidence	Potential mechanosensitive mediator; DO-specific evidence remains limited but is steadily growing.	(40,41,52)
RhoA-ROCK-YAP/TAZ axis	MSCs, osteoblast-lineage cells, suture stem cells	Regulates actin cytoskeleton organization, nuclear mechanotransduction, and transcriptional activation of osteogenic programs	<i>In vitro</i> mechanical loading and craniofacial/sutural DO studies	Supports mechanically induced osteogenesis and tissue remodeling	(14,36,38,39)
Wnt/ β -catenin signaling	Osteoblasts, MSCs, osteoprogenitor cells	Promotes osteoblast differentiation, matrix formation, and mechanical loading-induced bone formation	DO-specific animal studies and broader skeletal mechanobiology evidence	Strongly associated with distraction-induced bone regeneration	(38,84-87)
BMP-Smad signaling	Osteoblasts, MSCs, osteoprogenitor cells	Induces osteogenic differentiation and bone matrix formation; interacts with mechanical loading-induced transcriptional programs	Classical DO studies and mechanical strain models	Key osteogenic pathway activated during distraction	(15,24,29)
Akt-mTOR/NF- κ B signaling	Osteoblasts, MSCs, immune-responsive stromal cells	Links mechanical stimulation, cellular metabolism, inflammatory signaling, and osteogenic gene expression	<i>In vitro</i> mechanical loading studies and bone-regeneration evidence	Represents a molecular interface between mechanotransduction and inflammatory regulation	(29,83)
HIF-1 α /VEGF signaling	Osteoblasts, MSCs, endothelial cells	Couples mechanical stimulation and local hypoxia to angiogenesis and vascularized bone formation	DO-specific angiogenesis studies and bone-repair models	Central to angiogenesis-osteogenesis coupling during DO	(17,27,47,48)

DO, distraction osteogenesis; MSCs, mesenchymal stem cells; HIF, hypoxia-inducible factors; BMP, bone morphogenetic protein; YAP, Yes-associated protein; TAZ, transcriptional co-activator with PDZ-binding motif; FAK, focal adhesion kinase; Piezo1, piezo type mechanosensitive ion channel component 1; ROCK, Rho associated coiled-coil containing protein kinase; RhoA, ras homolog family member A.

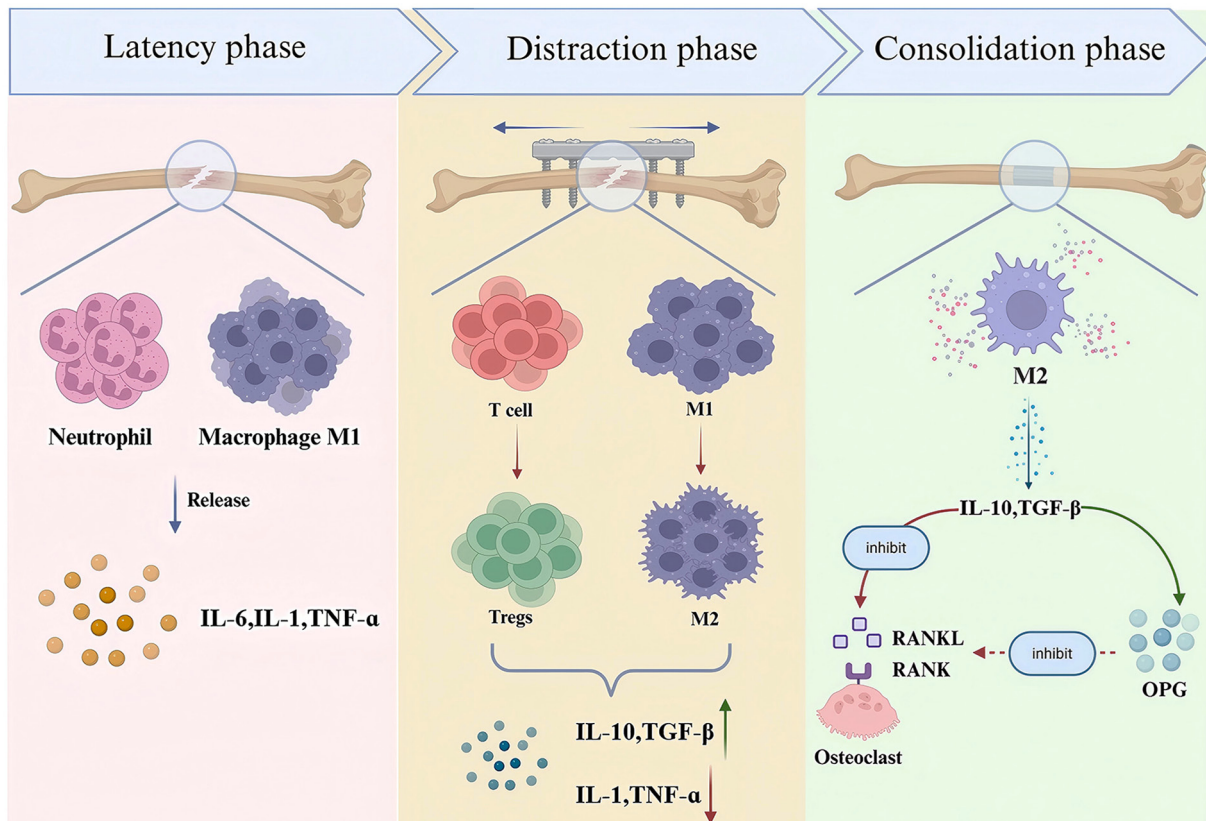


Figure 3. Immunological features during different phases of distraction osteogenesis. M2 macrophages provide essential cellular and molecular support for bone regeneration through the coordinated regulation of immune responses and tissue remodeling signals. These multifunctional contributions underscore the central role of immune-bone-vascular coupling in skeletal repair. Reprinted with permission (54). Copyright 2025, Frontiers in Bioengineering and Biotechnology. Tregs, T-regulatory cells; OPG, osteoprotegerin; RANKL, Rank ligand.

excessive acute inflammatory responses, accelerating the transition of the immune milieu to an M2-dominant reparative state, optimizing the osteogenic microenvironment (52,55). Notably, research on TNF signaling has demonstrated that direct intramembranous bone formation can occur in DO even in the absence of TNF signaling, when M1-mediated inflammation is diminished (25). This observation implies that under specific mechanical conditions, DO may partially bypass classical inflammation-dependent pathways and instead initiate bone formation through mechanotransduction-driven mechanisms.

Beyond macrophages, lymphocytes can also contribute to shaping the local immune landscape during DO. The balance among T-cell subsets exerts critical regulatory influence on bone regeneration. Type 1 T-helper (Th)1 cells inhibit osteogenesis through secretion of IFN- γ , whereas Th2 cells promote tissue repair through the production of IL-4 and related cytokines (56,57). Th17 cells secrete IL-17, which promotes osteoclastogenesis and amplifies inflammatory responses, thereby negatively impacting new bone formation (40). By contrast, regulatory T cells (Tregs) serve a protective role by secreting anti-inflammatory cytokines, such as TGF- β and IL-10, effectively dampening immune activation and supporting regenerative processes (11,58). Although data on T-cell dynamics in DO remain limited, accumulating evidence suggests that T-cell subsets undergo temporally coordinated changes during distraction. Mechanical forces may indirectly

regulate T-cell recruitment, activation and functional polarization, by modulating their antigen presentation or chemokine expression within the local microenvironment (59). A previous study has shown that periodic mechanical stress can induce periodontal ligament cells to promote the differentiation of Th17 cells into Tregs, thereby mitigating Th17-associated inflammation (60). Collectively, these findings indicate that within the unique biomechanical context of DO, mechanical signals can shape a balanced, repair-oriented immune microenvironment by modulating both the composition and functional states of immune cells, ultimately favoring successful bone regeneration.

The cytokine regulatory axis serves a pivotal role in bone regeneration during DO, with the temporal expression patterns of various inflammatory mediators closely coordinated with distinct phases of tissue repair. IL-6 levels increase during the early postoperative period and gradually decline as distraction is initiated, eventually stabilizing over time (16,45). IL-6 exhibits dual biological functions. It promotes the maturation of osteoprogenitor cells and supports osteogenic differentiation whilst also contributing to the inflammatory response. Its net effect is determined by the dynamic interplay between local concentration and temporal context. TNF- α is primarily secreted by M1 macrophages and other proinflammatory cells, such as neutrophils and activated T cells, in the early phase. At low to moderate concentrations, TNF- α can induce MSCs to differentiate toward osteogenic or chondrogenic

lineages. However, at high concentrations, it suppresses osteoblast activity and enhances bone resorption (61). In DO models characterized by direct intramembranous ossification, the functional contribution of TNF- α appears limited, whereas its regulatory role is more prominent in endochondral ossification-dependent processes, such as conventional fracture healing (25,62-64).

Similarly, IL-1 β contributes to early inflammation and fibroblast activation, but sustained high-level expression impedes bone formation. Preclinical studies have demonstrated that neutralization of IL-1 signaling can improve the mechanical integrity of healed fractures (65). Anti-inflammatory cytokines, such as IL-10 and TGF- β , are predominantly produced by M2-polarized macrophages and other immune cells, such as Tregs and Th2 cells, during the late latency and consolidation phases of DO. These mediators help suppress excessive inflammation and establish a microenvironment favorable for angiogenesis and osteogenesis (66,67). Collectively, a well-regulated, transient inflammatory phase is essential for successful DO. Adequate inflammatory signaling is required in the beginning to initiate repair, followed by a timely transition to an anti-inflammatory, reparative state. This dynamic shift is coordinately regulated by both mechanical cues and immune system feedback.

The immune regulatory axis also critically modulates osteoclast activity and coupling of bone remodeling. Osteoclasts originate from the monocyte/macrophage lineage, where their differentiation and activation are principally governed by the RANK ligand (RANKL)/RANK/osteoprotegerin signaling pathway (68). During the repair phase, moderate osteoclast activity facilitates the removal of immature woven bone and the structural refinement of trabecular architecture (69). However, excessive resorption compromises new bone volume and structural stability. Proinflammatory cytokines, such as TNF- α and IL-1 β , promote osteoclastogenesis by upregulating RANKL expression, whereas anti-inflammatory mediators, such as IL-10, exert inhibitory effects (51,68). Mechanical loading generally suppresses osteoclast activity (69), consistent with the physiological principle that mechanical stimulation favors bone formation over resorption (10,26). During the distraction phase of DO, persistent tensile stress may similarly restrain excessive bone resorption, thereby maintaining a bone-forming dominant environment. Multiple studies report that osteoclast activity in the distraction zone remains low during active lengthening, supporting efficient deposition of new bone matrix (15,69). Upon entering the consolidation phase, as mechanical stimulation diminishes, bone remodeling becomes more active and osteoclast numbers increase, initiating structural reorganization of the nascent bone. Histological analyses by Zaffe *et al* (70) in human mandibular DO biopsy specimens revealed that 88 days after cessation of distraction, osteoblastic activity declined whilst osteoclastic activity rose, accompanied by mild bone loss, which indicates a transition into the homeostatic remodeling phase. These findings underscore the critical role of the immune-osteoclast regulatory axis in coordinating bone remodeling. Once sufficient new bone has formed, the osteoclast-osteoblast coupling mechanism drives its transformation into mature lamellar bone. Immunologically, this phase is marked by resolution

of inflammation and dominance of regulatory immune signals, culminating in a dynamic equilibrium between bone formation and resorption.

Vascular-bone coupling represents a critical component of the immune interaction axis, as immune cell trafficking is highly dependent on the circulatory system, where newly formed blood vessels provide essential nutritional support and cellular transport routes for bone formation. As demonstrated by Amir *et al* (17), new bone volume in DO is significantly correlated with local microvascular density, with slower distraction rates associated with higher vascular preservation. VEGF, a key mediator induced by both hypoxic and inflammatory microenvironments, serves a central role during DO. It is secreted by inflammatory cells, such as macrophages and T cells, whilst mechanical stretch-induced tissue hypoxia further upregulates its expression (71). VEGF-driven angiogenesis enhances local perfusion, thereby facilitating osteogenesis and establishing a positive feedback loop between vascularization and bone regeneration. Notably, a specialized subset of functional blood vessels has been identified in bone regeneration that are referred to as 'type H vessels'. These vessels are characterized by CD31^{hi} endomucin (EMCN)^{hi} markers (72-74). Endothelial cells within these vessels promote osteogenic differentiation through the secretion of factors, such as platelet-derived growth factor (PDGF)-BB, directly linking angiogenesis to bone formation. Although research on type H vessels has primarily focused on fracture healing and osteoporosis models, accumulating evidence indicates that mechanical stress in DO may stimulate their formation through macrophage-derived signaling molecules, including VEGF and TGF- β , enhancing regenerative capacity (75,76). A previous study demonstrated that total flavonoids of *Rhizoma Drynariae* can significantly increase the abundance of CD31^{hi}Emcn^{hi} vessels and enhance vascular-osteogenic coupling in a rat model of DO, with this effect mediated through the PDGF-BB/VEGF/RUNX2 signaling axis (77). These findings suggest that targeting immune-associated vascular regulators may represent a promising therapeutic strategy to enhance distraction osteogenesis.

Overall, the immune interaction axis contributes to the entire course of DO through multiple coordinated mechanisms, including modulation of the inflammatory-anti-inflammatory balance, mediation of crosstalk between immune and osteogenic cells and promotion of neovascularization (Table II). Although distinct from the mechanotransduction axis, the immune axis exhibits extensive interplay with it. Mechanical forces shape the magnitude and temporal profile of inflammation, whereas immune-derived mediators reciprocally influence tissue responsiveness to mechanical stimuli and regenerative potential. A comprehensive understanding of the biological mechanisms underlying DO requires the integration of both mechanical and immune regulatory axes into a unified, dynamic and multilayered regulatory framework that captures the closed-loop nature of skeletal regeneration.

5. A mechanical-immune regulatory framework in DO

Mechanical stimulation and immune regulation are not independent biological events during DO but are dynamically interconnected throughout the regenerative process.

Table II. Immune and vascular regulators during different phases of DO.

DO phase	Main immune or vascular component	Representative mediators	Biological role	Evidence interpretation	(Refs.)
Latency phase	Neutrophils	ROS, proteases, inflammatory cytokines	Early debris clearance, antimicrobial defense, initiation of sterile inflammation	Supported by general bone injury and early repair biology; DO-specific temporal data remain limited	(22,23,50)
Latency phase	M1-polarized macrophages	TNF- α , IL-1 β , IL-6, iNOS	Clearance of necrotic tissue, inflammatory initiation, recruitment of reparative cells	Important for early repair, but excessive or persistent activation may impair regeneration	(16,50,54,79)
Latency to distraction phase	MSCs and osteoprogenitor cells interacting with immune cells	TGF- β , BMPs, IGF-related factors, chemokines	Cell recruitment, proliferation, osteogenic priming, and early matrix formation	Strong relevance to DO; interaction with immune cells requires further mechanistic clarification	(53,67,80)
Distraction phase	M2-polarized macrophages	IL-10, TGF- β , VEGF, BMP-related mediators	Resolution of inflammation, angiogenesis promotion, osteoblast differentiation, matrix remodeling	Increasing evidence supports a pro-regenerative role during mechanically induced bone formation	(14,52,67, 78,81)
Distraction phase	T-cell subsets	IFN- γ , IL-4, IL-17, IL-10, TGF- β	Regulation of inflammatory tone, osteoclastogenesis, and immune balance	Mechanistically plausible; direct DO-specific evidence is less extensive than macrophage-related evidence	(12,58-60)
Distraction phase	Endothelial cells and angiogenic vessels	VEGF, PDGF-BB, HIF-1 α -related mediators	Neovascularization, nutrient supply, endothelial-osteogenic coupling	Strong evidence supports angiogenesis as a key determinant of regenerate quality	(17,27,47,48)
Distraction and consolidation phases	Osteoclast lineage cells	RANKL, RANK, OPG, M-CSF	Bone resorption, removal of immature tissue, remodeling of woven bone	Essential for consolidation-stage remodeling and mature bone architecture	(68-70)
Consolidation phase	Osteoblast-osteoclast coupling system	RANKL/OPG balance, TGF- β , matrix-derived factors	Mineralization, lamellar remodeling, structural maturation, and integration with host bone	Supported by DO histological and bone-remodeling studies	(68-70)
Cross-phase regulation	Type H vessels and vascular-osteogenic niches	CD31hiEMCNhi markers, PDGF-BB, VEGF	Specialized angiogenesis-osteogenesis coupling and osteoprogenitor support	Emerging relevance to DO; strongest evidence derives from broader bone-regeneration studies	(72-77)

DO, distraction osteogenesis; ROS, reactive oxygen species; iNOS, inducible nitric synthase; RANKL, Rank ligand; OPG, osteoprotegerin; PDGF, platelet-derived growth factor; M-CSF, monocyte colony-stimulating factor; HIF, hypoxia-inducible factors; BMP, bone morphogenetic protein.

Mechanical cues directly activate mechanosensitive signaling pathways in osteogenic and stromal cells, while also influencing the local immune microenvironment through the regulation of inflammatory intensity, macrophage polarization, cytokine release, vascular remodeling and osteoclast-osteoblast coupling. By contrast, immune-derived cytokines, growth factors and matrix-remodeling mediators can all modulate the responsiveness of resident cells to mechanical stimuli, thereby affecting the efficiency, direction and maturation of mechanically induced bone formation. Based on these reciprocal interactions, DO can be understood as a mechanical-immune regulatory process involving mechanical input, mechanotransduction, immune microenvironment remodeling, vascularized osteogenesis and consolidation-stage feedback remodeling.

Mechanical regulation of the immune microenvironment. Upon application of tensile force, the mechanical state of local tissue is altered, leading to concomitant adjustments in cellular damage extent and the pattern of sterile inflammation. Compared with acute immobilized fractures, the gradual distraction in DO generates a low-intensity, sustained damage signal, which elicits a chronic, low-grade inflammatory response rather than a pronounced acute inflammatory peak (23,50). Consequently, mechanical stimulation modulates the kinetic profile of inflammation, steering the immune response toward a regenerative phenotype. Sustained mechanical strain promotes the accelerated phenotypic transition of macrophages from the proinflammatory M1 to the pro-reparative M2 subtype, facilitating earlier entry into the tissue remodeling phase. Experimental evidence indicates that compared with acute bone injury models, DO exhibits lower peak levels of proinflammatory cytokines and earlier resolution of the inflammatory phase (16,45,50). Furthermore, mechanical stretch exerts direct effects on immune cells, such as macrophages, modulating their transcriptional programs. *In vitro* studies have demonstrated that cyclic mechanical stress can downregulate the expression of inflammatory mediators iNOS, TNF- α and IL-6 in macrophages, whilst upregulating the expression of tissue repair-associated factors TGF- β , IL-4, BMP-2, BMP-4, IL-10 and VEGF (78-80). Furthermore, osteoblasts themselves respond to mechanical stimulation by secreting chemokines that contribute to immune cell recruitment and functional polarization. Mechanically stimulated osteoblasts release chemokines, such as C-C motif chemokine ligand 2, promoting monocyte migration to the distraction zone. Concurrently, osteoblast-derived TGF- β induces the differentiation of recruited monocytes into M2 macrophages (67,81). Collectively, mechanical forces can orchestrate immune responses through multiple interconnected pathways, thereby establishing a microenvironment that is highly conducive to *de novo* bone formation.

Immune modulation of mechanically induced osteogenesis. Immune cells and their secreted factors can profoundly influence the biological response of bone tissue to mechanical loading. Moderate infiltration of M2 macrophages enhances the osteogenic responsiveness of MSCs to mechanical stimulation through the secretion of growth factors, such as VEGF and BMP-2 (67). Previous evidence from periodontitis-associated

bone defect models has demonstrated that exogenous administration of M2 macrophages can significantly potentiate mechanically induced osteogenesis (82). By contrast, an excessively inflammatory local immune environment may lead mechanical stimuli to activate osteoclastic resorption or pro-fibrotic signaling pathways, thereby suppressing bone formation. Elevated levels of TNF- α and IL-1 have been shown to impair osteoblast responses to tensile strain, promoting fibrous tissue deposition or chondrogenic metaplasia rather than direct osteogenesis (65). Therefore, efficient transduction of mechanical signals into osteogenic outcomes is contingent upon a tightly regulated, repair-oriented immune milieu. This mechanistic insight explains the clinical contraindication of initiating DO in the context of active infection or ongoing inflammation. When mechanical distraction is applied in the setting of bacterial colonization or chronic inflammation, outcomes frequently include fibrous non-union or pseudoarthrosis, primarily due to aberrant immune-mediated negative feedback that counteracts or reverses the pro-osteogenic effects of mechanical stimulation (50,56).

Shared molecular hubs linking mechanical and immune signals. The mechanical-immune closed-loop regulation enables bidirectional crosstalk and systemic integration through shared key signaling nodes, including Akt/mTOR/NF- κ B, Wnt/ β -catenin and ROS-related signaling pathways. The Akt/mTOR/NF- κ B pathway represents a prototypical signaling nexus (29). Mechanical stress activates the Akt/mTOR axis, thereby promoting osteogenic differentiation. By contrast, NF- κ B signaling, as the central mediator of the canonical inflammatory signaling cascade, is modulated by the immune microenvironment. Under mechanical tension, mTOR and NF- κ B engage in a mutually reinforcing positive feedback loop that sustains the expression of osteogenesis-related genes. However, excessive inflammatory activation leading to hyperactivation of NF- κ B may suppress the mTOR pathway by negative feedback, attenuating the osteogenic response driven by mechanical stimulation (29,83). Conversely, at physiological levels, NF- κ B activity contributes positively to osteogenesis. This illustrates that mechanical and inflammatory signals do not function in isolation within cells but are functionally integrated through shared molecular pathways, collectively shaping cellular fate decisions. Additional critical nodes of cross-regulation include the Wnt/ β -catenin signaling pathway (84-87). Mechanical loading promotes β -catenin nuclear translocation, resulting in the activation of osteogenic gene programs. However, inflammatory mediators released by T cells, such as dickkopf Wnt signaling pathway inhibitor 1, can antagonize Wnt signaling and disrupt osteogenic progression. Furthermore, the oxidative stress pathway participates in this regulatory circuitry. Mechanical traction-induced tissue hypoxia can also generate reactive oxygen species (ROS). At moderate concentrations, ROS serve as secondary messengers that can facilitate osteogenic differentiation (88,89). By contrast, excessive ROS accumulation will induce oxidative damage to amplify inflammatory responses, ultimately impairing cellular function. Therefore, antioxidant mechanisms serve an essential homeostatic role in this closed-loop system, with their activity being tightly regulated by immune processes, particularly through macrophage-mediated secretion of antioxidant enzymes, such

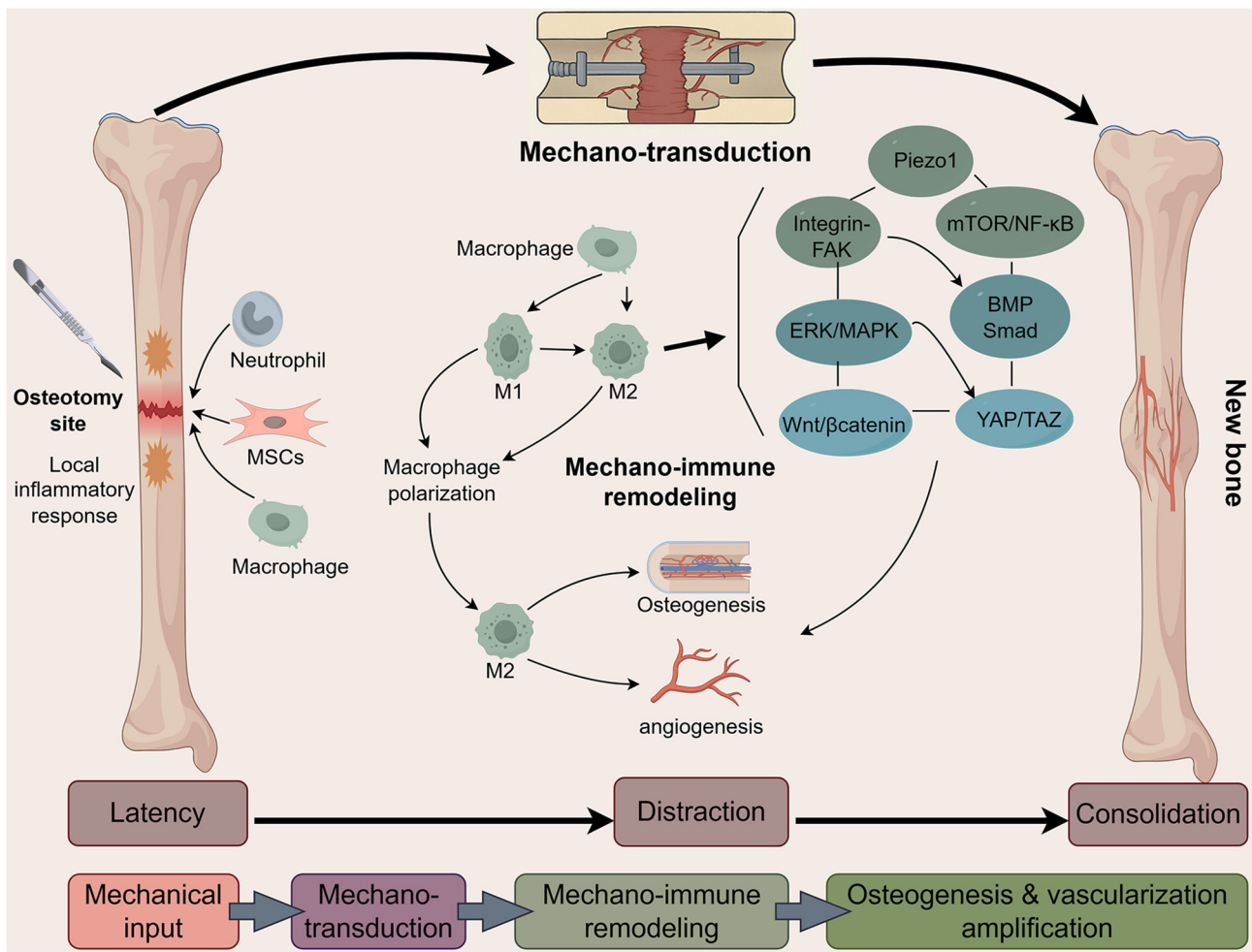


Figure 4. Mechanical-immune feedback regulation in DO. Controlled tensile loading provides the primary mechanical input during DO and activates mechanotransduction pathways in osteogenic, stromal, endothelial and immune-responsive cells. These pathways promote osteogenic differentiation, matrix deposition, angiogenic signaling and vascular remodeling. In parallel, mechanical stimulation modulates the local immune microenvironment by influencing inflammatory intensity, macrophage polarization, cytokine balance, osteoclast-osteoblast coupling and angiogenesis-related immune signals. Immune-derived mediators, including $\text{TNF-}\alpha$, $\text{IL-1}\beta$, IL-6 , IL-10 , $\text{TGF-}\beta$, VEGF, BMP-related signals and RANKL/OPG-associated remodeling cues, in turn regulate cellular mechanosensitivity, vascularized osteogenesis and consolidation-stage remodeling. This framework illustrates DO as a dynamic regulatory process in which mechanical input, mechanotransduction, immune remodeling, angiogenesis-osteogenesis coupling and bone remodeling interact across different phases of regeneration. OPG, osteoprotegerin; RANKL, Rank ligand; DO, distraction osteogenesis; BMP, bone morphogenetic protein; YAP, Yes-associated protein; TAZ, transcriptional co-activator with PDZ-binding motif; FAK, focal adhesion kinase; MSC, mesenchymal stem cell; Piezo1, piezo type mechanosensitive ion channel component 1.

as superoxide dismutase, which scavenge excess ROS and preserve microenvironmental stability (90).

Integrated feedback regulation during DO. The closed-loop regulatory logic is also manifested in the coordinated interplay of positive and negative feedback mechanisms across distinct regenerative phases. During the early distraction phase, mechanical stimulation serves as the primary driver of tissue regeneration, whilst a controlled inflammatory response acts as an auxiliary signal that establishes a pro-repair positive feedback loop. In the late distraction and consolidation phases, as mechanical input diminishes, osteogenic activity progressively shifts toward maturation and structural remodeling. At this stage, the immune system contributes to final bone morphogenesis through the secretion of reparative mediators and the activation of osteoclast-mediated bone turnover, thereby forming a negative feedback mechanism that prevents excessive or aberrant tissue formation (Fig. 4) (50,67,69,70).

Disruption of this closed-loop regulation leads to impaired regeneration. Insufficient or interrupted mechanical stimulation may shift the local immune microenvironment toward a chronic inflammatory state, favoring fibrotic healing over functional bone restoration. By contrast, excessive suppression of the immune response can attenuate mechanically induced osteogenesis due to impaired angiogenesis and deficiency in critical growth factors (50,66,67). Therefore, conceptualizing DO as a mechanically and biologically coupled closed-loop system provides a coherent explanation for diverse clinical observations. Patients with malnutrition or diabetes exhibit higher rates of postoperative complications following DO, attributable to systemic immune dysregulation, which indicates that peripheral immune status can modulate local bone regenerative capacity through a closed-loop mechanism (23). Similarly, prolonged use of non-steroidal anti-inflammatory drugs (NSAIDs) during DO suppresses early inflammatory signaling and is frequently associated with inadequate callus

formation, demonstrating that interference with immune components can destabilize the dynamic equilibrium of the entire regenerative circuit (91,92).

Taken together, the mechanical-immune regulatory perspective provides a coherent framework for interpreting DO as a dynamic and adaptive regenerative process rather than a purely mechanical or linear osteogenic event. Mechanical loading initiates and sustains osteogenic signaling, whilst immune cells and their mediators refine the inflammatory, vascular and remodeling microenvironment required for successful bone regeneration. In addition, the immune status of the local and systemic environment can influence whether mechanical cues are translated into vascularized bone formation, fibrous repair, delayed union or pathological remodeling. This integrated view may help explain the heterogeneity in DO outcomes and supports the development of phase-specific strategies that coordinate mechanical protocol optimization with biological and immunomodulatory interventions.

6. Commonalities and differences across anatomical sites and disease contexts

The biological principles of DO exhibit both conserved features and context-dependent variations across anatomical sites and disease states. In general, DO relies on a shared regenerative sequence involving inflammatory initiation, mechanically driven tissue formation, vascular remodeling, osteogenic differentiation and consolidation-stage remodeling (93-95). These core events are observed across appendicular long-bone, mandibular, craniofacial and sutural distraction models. However, the relative contribution of each component within the mechanical-immune regulatory process may differ substantially according to embryologic origin, local tissue architecture, marrow composition, vascular supply, fixation strategy, age and systemic metabolic or inflammatory status. Therefore, evidence derived from one DO context should be interpreted with attention to model specificity rather than directly generalized to all forms of DO.

Anatomical site differences (axial bone vs. craniofacial bone). Craniofacial bones, such as the maxilla and mandible, exhibit marked differences from appendicular long bones in terms of embryonic origin, bone marrow content, vascular supply patterns and tissue architectural composition (96). Craniofacial bones are primarily derived from neural crest cells and undergo intramembranous ossification as the dominant mode of bone formation, with limited development of medullary cavities (97). By contrast, long bones are originated from mesodermal precursors and feature abundant bone marrow compartments along with well-defined regions of endochondral ossification (98). As a result, during craniofacial distraction osteogenesis, excessive cartilage proliferation is rarely observed and new bone formation proceeds almost exclusively through intramembranous pathways under mechanical distraction (95). Conversely, in tibial or femoral DO, suboptimal mechanical conditions frequently lead to the formation of cartilaginous metaplasia or fibrous tissue at the distraction gap. This indicates that the mechanistic ‘coding threshold’ for optimal osteogenic response may be bone type-dependent. Mandibular DO typically

achieves robust bone regeneration at a distraction rate of 0.5-1.0 mm per day, whereas the tibia, although capable of tolerating higher distraction rates, is more susceptible to chondrogenic differentiation under accelerated protocols using distraction rates above the conventional 1.0 mm/day rate. Savoldi *et al* (13) highlighted that cranial suture distraction differs from long-bone DO in both biomechanical characteristics and biological responses, as craniofacial sutures are fibrous interfaces whose mechanical behavior varies with suture morphology, age and loading conditions, while craniofacial regeneration is predominantly intramembranous and long-bone DO may show a greater endochondral component under suboptimal mechanical conditions.

Influence of specialized tissue architecture. The skull and facial bones possess unique anatomical features, including cranial sutures and distinct MSC subpopulations, such as Gli1⁺ suture-derived stem cells (31,99). Ransom *et al* (9) demonstrated in a murine maxillary DO model that mechanical forces can induce the fate reprogramming of suture-resident stem cells, enabling them to reacquire a neural crest-like multipotent state, thereby accelerating bone regeneration. This phenomenon, termed ‘mechanically induced re-development’, has not been reported in long bone DO, suggesting that craniofacial bones may harbor specialized stem cell regulatory mechanisms in response to mechanical stimuli. Furthermore, the facial region is characterized by dense vascular networks and a relatively thin overlying soft tissue envelope, which collectively shape the local mechanical and biological microenvironment during distraction (93,96). Whilst the abundant vascularity supports rapid immune cell recruitment and enhances regenerative potential, it also predisposes the site to a higher risk of infection (93,100). Clinically, implantable distraction devices are commonly employed in craniofacial DO to minimize infection risks. By contrast, limb DO typically relies on external fixators, which are susceptible to pin-site infections that may trigger localized immune activation and disrupt normal osteogenesis (100). A comparative study on pediatric and adult patients undergoing limb lengthening with magnetic intramedullary lengthening nails revealed that although internal lengthening systems can eliminate complications related to external fixation, excessive elongation ratios (>90%) are associated with increased fracture risk and higher reoperation rates (101). These findings indicate that regardless of anatomical location, mechanical interventions exceeding the physiological adaptive capacity of tissues can provoke heightened stress responses, leading to immune dysregulation and impaired healing.

Growth status and age. Pediatric bones exhibit higher cellular activity and extracellular matrix plasticity, allowing for a moderately accelerated distraction rate during DO, whilst typically achieving superior quality of newly formed bone (95). Furthermore, the developing immune system in children supports enhanced regenerative capacity, characterized by a heightened propensity for M2 macrophage polarization, which promotes an anti-inflammatory and pro-regenerative microenvironment (102). By contrast, aging is associated with a decline in MSC quantity and differentiation potential, accompanied by immunosenescence, commonly referred to as ‘inflammatory aging’, which is manifested by chronically

elevated baseline inflammation (18,103). These age-related biological changes collectively contribute to delayed bone healing and an increased risk of complications following DO in elderly patients. Amir *et al* (18) reported in elderly individuals undergoing mandibular DO that those subjected to higher distraction rates exhibited reduced vascular density and inadequate bone formation, potentially attributable to diminished mechanosensitivity and impaired inflammatory regulation. Therefore, clinical management of DO in older adults should involve a reduced distraction rate, extended consolidation phase and consideration of adjunctive biological interventions, such as administration of bone growth factors, to augment osteogenic outcomes.

Disease states. Pathological conditions, such as osteoporosis, diabetes and post-radiation bone injury, significantly alter the biological mechanisms underlying DO. In osteoporosis, reduced bone mass and an imbalance between osteogenic and osteoclastic activity are commonly accompanied by a chronic low-grade inflammatory immune microenvironment. Preclinical studies in osteoporotic animal models have demonstrated delayed mineralization of newly formed bone during DO, frequently necessitating prolonged consolidation periods or adjunctive pharmacological interventions (104). Omi *et al* (105) showed in a rabbit model that localized administration of low-dose bisphosphonates can enhance bone mineral density and mechanical integrity within the distraction zone, thereby partially offsetting deficits in bone volume. In diabetic conditions, persistent hyperglycemia can induce chronic inflammation and microvascular dysfunction, both of which can impair osteoblast function and hinder bone regeneration (106,107). Evidence supports improved healing outcomes through strict glycemic control combined with pro-angiogenic therapies to restore vascular support (106,107). In irradiated bone, severe vascular compromise and markedly diminished MSC viability render conventional DO protocols ineffective. Successful bone formation in such cases typically requires multimodal strategies, including stem cell transplantation or systemic delivery of vasoactive agents, such as erythropoietin and norepinephrine, to promote neovascularization and enhance regenerative capacity (108-111). Collectively, these findings underscore that preexisting medical conditions can disrupt the dynamic interplay between mechanical stimulation, immune regulation and angiogenesis, which are key components of the mechanobiological-immune axis, thereby mandating personalized treatment approaches tailored to the patient's pathophysiological context.

Deformity types. Patients with congenital skeletal dysplasias, such as achondroplasia and osteogenesis imperfecta, exhibit altered osteocyte function and distinct immune profiles compared to healthy individuals. Blount's disease, a metabolic bone disorder characterized by progressive tibial varus deformity, is associated with impaired bone formation capacity during DO, necessitating a combined approach that integrates corrective osteotomy with metabolic management (112,113). Clinical reports have demonstrated the efficacy of a staged protocol for correcting lower limb deformities in Blount's disease. This protocol involves initial high-magnitude distraction to harness mechanical stimulation for bone regeneration,

followed by external fixation to maintain alignment and ensure proper load-axis correction (112,114). In these complex cases, the mechanobiological-immune axis may be inherently compromised due to underlying pathophysiology, thereby requiring exogenous support to achieve successful bone regeneration. Patients with osteogenesis imperfecta suffer from defective collagen synthesis in osteoblasts and frequently exhibit a chronic proinflammatory immune state, both of which contribute to suboptimal outcomes in DO. Future therapeutic strategies may involve integrated approaches combining gene-based therapies with targeted immunomodulation to restore regenerative potential and improve clinical efficacy (115).

Across these contexts, DO is unified by its dependence on controlled mechanical traction and the intrinsic regenerative capacity of skeletal tissues. However, the biological response to distraction is shaped by site-specific and patient-specific factors. Craniofacial and pediatric settings may exhibit stronger cellular plasticity, favorable vascularity and more efficient repair responses (95,96,102), whereas elderly individuals and patients with osteoporosis, diabetes, radiation injury, chronic inflammation or congenital skeletal disorders may show impaired mechanosensitivity, altered immune regulation, compromised angiogenesis or delayed remodeling (18,103,104,106-108,115). Recognizing these differences is essential for interpreting experimental findings across models and for designing individualized DO protocols. In clinical practice, such heterogeneity supports careful adjustment of distraction rate, rhythm, latency period, consolidation duration, fixation strategy and adjunctive biological interventions according to anatomical location and patient condition.

7. Therapeutic strategies and clinical translation

Building on the aforementioned mechanical-immune regulatory framework, therapeutic optimization in DO may be approached from two complementary directions: i) Refinement of mechanical protocols; and ii) modulation of the biological microenvironment (5,26,54). Mechanical strategies aim to improve the timing, magnitude, rhythm and stability of distraction forces, whereas biological strategies seek to support angiogenesis, osteogenesis, immune balance and remodeling within the distraction gap. Rather than functioning as isolated interventions, these approaches may be most effective when applied in a phase-specific and context-dependent manner according to anatomical site, patient condition and regenerate maturity.

Mechanical strategy optimization primarily involves the precise modulation of distraction parameters and the integration of auxiliary physical stimuli (19,28). While conventional clinical protocols have established standard regimens, such as a daily distraction rate of 0.5-1 mm and a latency period of ~7 days for adult long bones, substantial inter-individual variability necessitates personalized parameter selection (18,116). Advances in numerical modeling and preclinical animal studies have provided a scientific foundation for refining these parameters (116-119). Liu *et al* (120), through computational simulation, demonstrated that early application of transient alternating tensile and compressive forces, known as the accordion technique,

can enhance osteogenesis within an optimal intensity and frequency range. However, exceeding these thresholds may induce inhibitory effects on bone formation. With regards to adjunctive physical modalities, low-intensity pulsed ultrasound has been validated as an effective intervention for promoting bone tissue maturation (121). In a rabbit DO model, Shimazaki *et al* (28) reported that daily LIPUS exposure during the consolidation phase significantly increased hard callus area, bone mineral density and biomechanical strength compared with controls. Notably, even under accelerated distraction conditions without a latency period, LIPUS facilitated the mineralization of immature bone tissue. This non-invasive approach has undergone limited but growing clinical adoption, particularly in cases of delayed union or impaired healing (122,123). Another promising direction is the development of load-capable internal fixation systems, including devices embedded with force sensors or capable of inducing controlled micro-motion, which help sustain anabolic mechanical cues during consolidation and mitigate callus atrophy caused by stress shielding (19,124). The use of magnetic intramedullary lengthening nails represents a major advancement by eliminating external fixators entirely, thereby enhancing patient mobility and restoring a more physiological mechanical environment for surrounding soft tissues, which contributes to a marked reduction in complication rates (101). In the future, distraction systems integrating imaging-based assessment, biosensor feedback and force-monitoring technologies may enable the more dynamic adjustment of distraction parameters according to the extent of regenerative maturation. Although such approaches remain largely developmental, they represent a promising direction for translating mechanical-immune feedback concepts into more individualized and adaptive DO management.

Biological interventions primarily target the immune interaction axis and key osteogenic signaling pathways, with multiple approaches currently under investigation. A central focus lies in cell-based therapies and the localized delivery of bioactive molecules. The extended temporal window inherent to the DO process offers a favorable opportunity for exogenous cellular or molecular modulation. Previous research on MSC therapy has shown that the local administration of conditioned medium from human fetal MSCs into the distraction gap of rats significantly accelerated bone bridging and improved the mechanical integrity of newly formed bone (125). The authors attributed these effects to the low immunogenicity and high concentration of pro-osteogenic factors in fetal MSC secretions, which may recapitulate an M2-like immunomodulatory microenvironment and synergistically support bone regeneration. A previous study explored endothelial progenitor cell-derived exosomes, which promote angiogenesis and thereby indirectly couple vascular and bone regeneration (126). These nanoscale biologics carry diverse growth factors and regulatory microRNAs, enabling dual functionality, such as modulating immune cell behavior and directly stimulating osteoblast activity, which are regarded as promising multifunctional therapeutic platforms. In the realm of growth factor delivery, BMP-2 and BMP-7, members of the BMP family, are widely utilized in orthopedic applications. However, their use in DO requires stringent control over dosage and timing due to

the risk of adverse outcomes, such as heterotopic ossification or excessive callus formation (127,128). Given that mechanical loading naturally upregulates endogenous BMP expression, supplementation with high-dose exogenous BMP may disrupt the physiological balance. By contrast, combinatorial strategies involving angiogenic and immunomodulatory factors show greater promise, such as co-application of PDGF-BB and VEGF, which can recreate a pro-regenerative microenvironment conducive to early vascular remodeling (129). Preclinical evidence further supports this approach, as genetically engineered bone marrow mesenchymal stem cell sheets with sustained BMP-1 expression have been shown to significantly enhance osteogenic efficiency in DO models (130).

Immunomodulatory therapies represent a potential direction for enhancing DO. However, their timing, dosage and biological context require careful consideration. Short-term anti-inflammatory treatment may be necessary for postoperative pain control and excessive acute inflammation, but prolonged or non-selective suppression of inflammatory signaling may interfere with the early repair responses required for angiogenesis and osteogenesis. Therefore, NSAID use during DO should be interpreted in a dose- and phase-dependent manner rather than as uniformly beneficial or harmful (131,132). Similarly, although experimental findings suggest that TNF- α signaling may be less necessary for direct intramembranous bone formation during DO than for conventional endochondral fracture repair, systemic TNF inhibition should not be regarded as a routine strategy for improving DO outcomes (25). Its potential value, if any, may be limited to selected hyperinflammatory conditions, which requires rigorous validation in DO-specific models and clinical studies. Novel targeted approaches, such as localized delivery of IL-10 or strategies that promote a timely transition from M1-dominant inflammation to M2-associated repair, may offer a more physiologically compatible form of immune modulation (133). Antibiotics are routinely administered during the perioperative period of DO primarily to prevent infection. From an immunoregulatory standpoint, they also serve to minimize aberrant immune activation by microbial components, maintaining bone regeneration within a controlled, sterile inflammatory framework (56). There is an increasing interest in modulating the systemic immune status. Previous evidence indicates that moderate physical activity under physiological mechanical loading enhances systemic immunity and promotes the mobilization of peripheral MSCs and endothelial progenitor cells (134,135). For patients undergoing prolonged DO treatment, structured exercise programs and nutritional supplementation, such as vitamin D and polyunsaturated fatty acids, known for their anti-inflammatory properties, may contribute to sustaining the dynamic equilibrium of the mechanical-immune regulatory loop.

Advances in materials science and drug delivery systems have further strengthened the therapeutic potential of DO. The use of biodegradable magnesium or its alloys as internal scaffolds offers dual advantages, namely initial mechanical stability and bioactive ion release. During degradation, magnesium ions exert both immunomodulatory and osteo-inductive effects (136). Li *et al* (137) demonstrated that magnesium-based implants in DO models can significantly

enhance new bone formation. This effect is mediated by the magnesium-induced stimulation of sensory nerve endings, leading to the release of calcitonin gene-related peptide, which subsequently activates the FAK/VEGF signaling pathway to coordinately promote angiogenesis and osteogenic differentiation. This approach exemplifies an integrative strategy combining mechanical support, biomaterial functionality and immunobiological regulation. Furthermore, small-molecule therapeutics offer precise targeting of critical nodes within the mechanobiological-immune circuit. RhoA agonists can potentiate YAP-mediated mechanotransduction, whereas mTOR activators can amplify the osteogenic response to mechanical stimuli (36). Despite their promise, clinical translation of these agents requires careful risk-benefit assessment to ensure that systemic homeostasis is preserved and unintended disruptions, such as pathway overactivation leading to regulatory imbalance, are avoided.

The application of digital medicine and regenerative status assessment technologies is gaining increasing importance in the field of DO. Emerging directions focus on real-time monitoring of mechano-biological responses throughout the regeneration process (19,119). By leveraging radiomics analysis or serum and tissue biomarker profiling, clinicians can dynamically assess callus mineralization and inflammatory activity, enabling personalized adjustments to the distraction protocol or timely initiation of adjunctive interventions (16,54,138). Smart fixation systems integrated with sensors allow continuous, real-time tracking of inter-segmental micromotions and axial forces. When combined with systemic inflammatory markers as physiological feedback signals, these data provide a robust foundation for closed-loop control of bone regeneration (19). Concurrently, tissue engineering approaches offer promising avenues for enhancing DO outcomes. Pre-implantation of three dimensional (3D)-printed scaffolds seeded with osteo-progenitor cells and immunomodulatory factors into the distraction gap enables progressive replacement by newly formed bone during lengthening (139,140). Such engineered constructs function as ‘enhancement modules’ within the mechanical-immune regulatory circuit, augmenting intrinsic regenerative capacity from the outset. In anatomical regions requiring high morphological precision, such as the craniofacial skeleton, DO can be strategically integrated with reconstructive surgical techniques. This combined approach first utilizes distraction to generate adequate bone volume, followed by precise contouring to achieve optimal functional and aesthetic restoration (141). Collectively, these strategies are grounded in an evolving understanding of mechanical-immune crosstalk and reflect a strong translational potential from mechanistic insights to clinical innovation.

To summarize, current therapeutic strategies are shifting toward coordinated modulation of both mechanical and immune axes, moving beyond isolated interventions targeting single pathways. This multi-dimensional, system-level approach holds great promise for enhancing the efficacy and reliability of DO. Overall, therapeutic strategies for DO are evolving from single-factor enhancements towards coordinated regulation of mechanical parameters, vascularization, immune balance and bone remodeling. Future progress will depend on the rigorous validation of emerging interventions in DO-specific models and well-designed clinical

studies, with the goal of developing safer, phase-specific and patient-adapted strategies to improve regenerate quality and reduce treatment-related complications.

8. Limitations and future directions

Despite substantial progress made in understanding mechanotransduction, immune regulation, angiogenesis and bone remodeling during DO, several important limitations remain. Current evidence regarding mechanical-immune feedback regulation remains fragmented. The majority of previous studies have investigated mechanical signaling (142,143) or immune regulation separately (6,59,144), whereas direct experimental analyses integrating these two axes within the same DO model remain limited (55,67). Therefore, the proposed mechanical-immune regulatory framework should be interpreted as a synthesis of the available evidence and as a basis for hypothesis generation, rather than as a fully resolved causal network. Future studies should combine DO-specific animal models, lineage tracing, intravital imaging, spatial transcriptomics and single-cell sequencing to characterize immune cell recruitment, macrophage polarization, osteogenic lineage progression, vascular remodeling and their temporal associations under defined mechanical conditions.

In addition, existing experimental models have inherent limitations. Rodent DO models and two-dimensional mechanical loading systems have provided valuable mechanistic insights, but they cannot fully reproduce the multicellular, vascularized, mechanically dynamic and immune-active environment of clinical DO (71,142,145-148). *In vitro* systems, such as Flexcell-based tensile loading platforms, allow controlled investigation of osteoblasts, MSCs or immune cells under defined strain conditions, but they lack the complex spatial organization, extracellular matrix remodeling, vascular perfusion and systemic immune inputs present *in vivo* (149). Conversely, large-animal models and clinical studies more closely mimic human DO, but are limited by cost, ethical considerations, sample heterogeneity and difficulty in obtaining serial tissue-level data (18,70,146). Future model development should therefore integrate 3D co-culture systems, organoid-like skeletal constructs, microfluidic platforms and mechanically tunable bioreactors to recapitulate the coupled mechanical, immune and vascular environment of the distraction gap more effectively.

Current understanding of the negative feedback regulatory mechanisms within the mechanical-immune feedback regulatory system also remains limited. Although a bidirectional regulatory association between mechanical and immune processes is recognized, it is still unclear under what specific pathological or physiological conditions one axis becomes the rate-limiting factor in regeneration. It needs to be determined when inflammation transitions from a supportive to a detrimental role that requires therapeutic intervention, and whether excessive mechanical stimulation can trigger specific immunosuppressive pathways. Addressing these questions demands more refined experimental approaches, such as targeted gene knockout or overexpression of key signaling molecules to assess their impact on regulatory balance. Furthermore, patient-specific factors, such as genetic background, age and sex, may

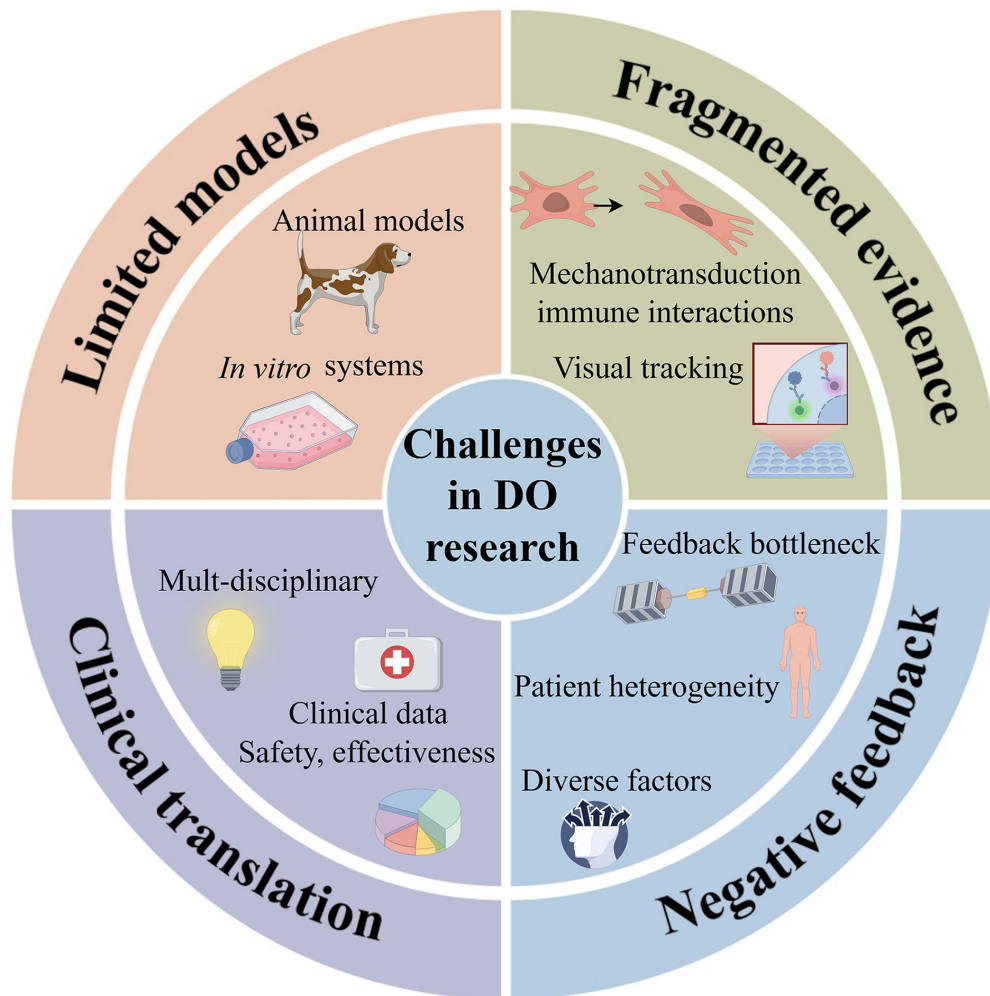


Figure 5. Current challenges and future directions in mechanical-immune feedback regulation during DO. Existing evidence remains fragmented because mechanotransduction and immune regulation have often been investigated separately. Direct studies integrating mechanical loading, immune cell dynamics, vascular remodeling, osteogenic lineage progression and consolidation-stage remodeling within the same DO model remain limited. Additional challenges include the limited ability of conventional *in vitro* systems and small-animal models to reproduce the multicellular, vascularized, mechanically dynamic and immune-active environment of clinical DO. Future studies should incorporate DO-specific animal models, lineage tracing, intravital imaging, single-cell and spatial omics, mechanically tunable bioreactors, computational modeling and prospective clinical biomarker monitoring to clarify causal relationships, temporal hierarchy and translational thresholds within the mechanical-immune regulatory network. DO, distraction osteogenesis.

significantly influence individual sensitivity to mechanical loading and patterns of immune response. Certain individuals may inherently exhibit robust mechano-induced osteogenesis, whereas others may be predisposed to maladaptive inflammatory reactions (18,54,103). Such inter-individual heterogeneity can be systematically investigated using multi-omics technologies and large-scale data analytics. In the future, pre-treatment profiling of patient genetic architecture and immune phenotypes could enable the prediction of bone healing capacity and inform personalized DO protocols. This shift necessitates a move in basic research from conventional population-level analyses toward individualized mechanistic studies, including the application of single-cell RNA sequencing to identify differences in mechano-responsive gene expression among MSCs and immune cell subsets across individuals (30,53,81).

Additionally, enhanced interdisciplinary integration is urgently needed. The investigation of DO mechanisms spans multiple domains, including biomechanics, cell biology, immunology, materials science and engineering, where

its advancement relies on coordinated efforts across these disciplines. Particularly, in model development and algorithm design, computational biology holds remarkable promise. Morgan *et al* (48) employed finite element modeling to examine the association between tissue strain distribution and cellular differentiation, establishing mathematical frameworks to quantitatively link mechanical inputs with biological outcomes. Building upon this foundation, future studies may develop more sophisticated multi-scale computational models that incorporate immune-related variables such as spatiotemporal gradients of inflammatory cytokines, immune cell migration and dynamic spatial distributions to simulate the integrated behavior of the mechanical-immune regulatory network. These models could serve as predictive tools for generating novel hypotheses and guiding experimental validation.

From a clinical translation perspective, high-quality clinical trial data remain sort after to validate the efficacy and safety of emerging intervention strategies. Currently, the majority of therapeutic approaches aimed at enhancing DO

healing remain confined to preclinical animal studies and lack systematic evaluation in human subjects (125,126,130,137). Key translational challenges include how to safely and controllably modulate patient immune status and how to achieve targeted delivery of biological agents within the distraction gap, questions that represent central hurdles in advancing these therapies to clinical practice (11,54,140). MSC-derived exosomes have demonstrated promising regenerative potential in animal models. However, challenges related to large-scale production, quality standardization, precise delivery and regulatory approval pathways remain unresolved (150,151). Future advances may depend on the development of localized sustained-release systems or biodegradable carrier technologies to enable efficient, stable and site-specific delivery of therapeutics or cell-derived factors. Concurrently, potential adverse effects must be carefully monitored, particularly the risk of infection associated with excessive immunosuppression, necessitating precise control over both dosage and timing of interventions. Clinical translation should proceed through a phased and evidence-based pathway, beginning with low-risk interventions, such as protocol optimization, non-invasive monitoring, nutritional support and localized delivery systems before advancing to more complex strategies involving cell-derived products, immunomodulatory drugs or sensor-guided adaptive devices. Such progression will require standardized outcome measures, long-term safety assessment and prospective clinical validation.

In conclusion, current research supports a broad mechanical-immune feedback perspective for interpreting DO. However, the causal sequence, temporal hierarchy and context-specific regulatory thresholds within this framework remain incompletely defined (Fig. 5). Advances in high-resolution imaging, single-cell and spatial omics, biomaterials, computational modeling and prospective clinical biomarker studies are expected to refine this framework and clarify how mechanical and immune signals jointly regulate skeletal regeneration. Such progress may not only improve the biological understanding of DO, but also provide transferable insights into mechano-immunological regulation in other regenerative contexts, including fracture repair, cartilage regeneration and tendon healing.

9. Conclusion

DO is a distinctive model of mechanically induced bone regeneration in which tensile loading, immune regulation, angiogenesis, osteogenesis and remodeling are temporally coordinated. Current evidence indicates that DO should not be interpreted solely as a mechanically driven process, but as a regenerative sequence shaped by the interaction between mechanotransduction and the evolving immune microenvironment (5,26,54,81). Mechanical cues are sensed by integrin/FAK complexes, primary cilia, mechanosensitive ion channels, cytoskeleton-associated nuclear signaling systems and downstream pathways, including MAPK, Wnt/ β -catenin, BMP/Smad, Akt/mTOR/NF- κ B and RhoA/YAP/TAZ. These signals promote osteogenic differentiation, extracellular matrix deposition and vascular remodeling. In parallel, immune cells and immune-derived

mediators, particularly macrophage polarization, cytokine balance, osteoclast-osteoblast coupling and angiogenesis-related signals, regulate the initiation, progression and maturation of the regenerate.

On this basis, mechanical-immune feedback regulation provides a coherent framework for understanding the biological complexity and clinical heterogeneity of DO across anatomical sites, developmental stages, aging and pathological conditions. This perspective supports phase-specific and patient-adapted strategies, including optimization of distraction protocols, biomarker-guided monitoring, localized immunomodulation, vascular-enhancing interventions and mechanically responsive biomaterials. Future studies integrating DO-specific models, single-cell and spatial omics, intravital imaging, advanced bioreactors and prospective clinical biomarker analyses are needed to clarify causal associations and context-dependent thresholds within this regulatory network. A more refined understanding of mechanical-immune feedback may facilitate safer and more precise approaches to enhance bone regeneration, shorten treatment duration and reduce complications in DO and related skeletal regenerative settings.

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Authors' contributions

XW conceptualized the review, curated the relevant literature, conducted literature synthesis and wrote the original draft. YW provided supervision, contributed to literature evaluation and interpretation, and critically revised the manuscript for important intellectual content. SH performed literature investigation and contributed to evidence synthesis and critical revision of the manuscript. CM administered the project, provided resources, contributed to literature interpretation and critically revised the manuscript for important intellectual content. ZH developed visualization components, contributed to the conceptual synthesis of the reviewed evidence and critically revised the manuscript for important intellectual content. XH acquired funding and was responsible for review and editing. All authors read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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