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Inflammation and bone healing; pathophysiological foundations of fracture and biological strategies for the treatment of nonunion

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Abstract

Fracture healing involves coordinated phases of inflammation, callus formation, and remodeling, regulated by complex interactions among immune mediators, bone cells, and vascular networks. Disruption of these processes can result in delayed union or nonunion, a significant orthopedic challenge. This review synthesizes current evidence on normal repair physiology, nonunion mechanisms, biomarkers, and emerging therapies. Key topics include immune cell and cytokine dynamics, extracellular matrix (ECM) deposition, angiogenesis, and osteoclast–osteoblast coordination; pathophysiological drivers of nonunion such as persistent inflammation, impaired osteogenesis, macrophage polarization imbalance, microvascular dysfunction, systemic risk factors, and characteristic radiological–histological profiles. The role of inflammatory, bone formation, and angiogenesis biomarkers, through gene expression and immunohistochemistry is examined, along with therapeutic strategies using growth factors, cell-based treatments, engineered scaffolds, and targeted immunomodulation. Comparative analyses of animal and in vitro models highlight their translational value and limitations, and evidence is discussed regarding conventional versus biologically driven approaches to optimize fracture repair outcomes.

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Introduction

Bone healing is a highly regulated biological process that restores skeletal integrity through coordinated mechanical, cellular, and molecular interactions. Following fracture, an immediate inflammatory response initiates the repair cascade and recruits progenitor cells essential for regeneration (1). While traditionally considered a transient event, inflammation critically influences the quality and speed of healing, with both insufficient and excessive responses contributing to delayed union or nonunion (2).

The process progresses through inflammatory, reparative, and remodeling phases, with the initial inflammatory phase establishing the framework for repair (3). Hematoma formation within hours provides a matrix rich in platelets and cytokines such as interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6), which recruit neutrophils and macrophages (4). These cells clear debris and release growth factors like vascular endothelial growth factor (VEGF) and transforming growth factor-beta 1 (TGF-β), promoting angiogenesis and mesenchymal stem cell (MSC) differentiation. Precise regulation is essential, as dysregulation can impair osteogenesis (5). Nonunion results from disrupted healing and may be driven by persistent inflammation, mechanical instability, infection, poor vascularity, or systemic factors (6). Chronic inflammation can suppress osteoprogenitor activity and matrix deposition, whereas inadequate activation may fail to trigger adequate repair (7).

Biological therapies targeting the inflammatory microenvironment include growth factors (8), Advances in molecular biology have also opened avenues for targeted therapies that influence MSC-based treatments, and immunomodulatory agents to guide macrophage polarization from M1 to M2 phenotypes (9).

In light of the central role of inflammation in bone repair, a comprehensive understanding of its temporal dynamics, molecular mediators, and cross-talk with osteogenic pathways is essential. By integrating pathophysiological insights with emerging biological therapies, clinicians and researchers can move toward

Key point

Fracture healing depends on precise immune–vascular–cellular coordination across distinct phases. Disruption causes nonunion by inflammatory dysregulation, cellular dysfunction, and impaired angiogenesis. This review integrates molecular, imaging, and biomarker evidence, elucidates mechanisms, and highlights emerging biologic therapies compared with conventional approaches, offering a comprehensive basis for improved orthopedic outcomes.

more effective, targeted solutions for preventing and treating nonunion, ultimately improving functional outcomes and quality of life for patients with fractures.

Materials and Methods

A comprehensive literature search was conducted to identify relevant publications addressing the role of inflammation in bone healing, the pathophysiology of fracture repair, and biological strategies for the treatment of nonunion. The electronic databases PubMed/MEDLINE, Scopus, Web of Science Core Collection, and Embase were systematically searched from database inception to July 2025, without language restrictions. The search strategy combined controlled vocabulary terms (MeSH and Emtree) and free-text keywords, adapted for each database, using Boolean operators. The primary search terms included "inflammation", "bone healing", "fracture healing", "nonunion", "delayed union", "pathophysiology", "biological therapy", "mesenchymal stem cell", "macrophage polarization", and "bone morphogenetic protein". These terms were conducted in various combinations with Boolean operators "AND" and "OR" to maximize sensitivity while maintaining relevance. Reference lists of eligible articles and pertinent reviews were manually screened to capture additional studies. Eligible publications included original experimental or clinical studies, systematic reviews, and relevant preclinical research investigating the interplay between inflammatory processes and bone repair or evaluating biological interventions aimed at enhancing fracture union.

Results

The inflammatory phase of fracture healing begins almost immediately after injury, driven by hematoma formation and rapid recruitment of immune cells that initiate the reparative cascade. In uncomplicated fractures, this phase generally resolves within three to seven days, but extensive soft-tissue damage, poor perfusion, or systemic comorbidities can prolong it well beyond this window. Initially, neutrophil infiltration and surges in pro-inflammatory cytokines such TNF-α and IL-1β dominate the first 24–48 hours (10).

As the process advances, macrophage populations undergo a critical phenotypic transition from the pro-inflammatory M1 state to the pro-healing M2 state, enabling angiogenesis, ECM deposition, and osteogenic activity. The efficiency of this temporal shift is strongly influenced by host factors, including age, metabolic disease, smoking, and corticosteroid use, which may delay resolution, whereas prompt fracture stabilization, adequate vascular supply, and infection control support timely progression toward bone formation. Effective modulation of these temporal dynamics represents a promising strategy to enhance repair and prevent nonunion (11).

Roles of immune cells (macrophages, neutrophils, lymphocytes)

Macrophages, neutrophils, and lymphocytes each exert distinct yet interdependent functions in fracture healing, collectively guiding the transition from inflammation to tissue regeneration. Macrophages exhibit functional plasticity, with pro-inflammatory M1 subsets dominating early after injury to amplify immune recruitment and clear debris, followed by a shift toward M2 phenotypes that release anti-inflammatory mediators and growth factors such as VEGF and TGF-β to promote angiogenesis and osteogenesis. Neutrophils, arriving within hours of fracture, deliver proteolytic enzymes, reactive oxygen species, and chemotactic signals essential for defense and monocyte attraction; however, their prompt clearance is vital to avoid excessive tissue injury and prevent chronic inflammation. Lymphocytes, though fewer in number, play key immunoregulatory roles—T cells influence macrophage polarization and balance osteoclastic and osteoblastic activity, regulatory T cells temper pro-inflammatory responses to preserve tissue homeostasis, and B cells contribute both antibody production and cytokine secretion with potential indirect effects on bone metabolism. The orchestrated timing and interplay of these immune cell populations establish a pro-repair microenvironment that is critical for successful fracture healing (12).

Key cytokines and mediators (TNF-α, IL-1β, IL-6, IL-10)

Cytokines act as pivotal molecular regulators of the fracture healing cascade, with their temporal expression dictating the balance between inflammation and repair. TNF-α is rapidly upregulated post-injury, promoting leukocyte recruitment by endothelial adhesion molecule expression and initiating osteoclast differentiation to clear damaged matrix, yet its sustained overproduction can impair healing and contribute to osteolysis and nonunion. IL-1β, working synergistically with TNF-α, enhances immune cell infiltration, drives matrix metalloproteinase activity, and induces COX-2–mediated prostaglandin E2 synthesis, amplifying vascular permeability; however, prolonged elevation disrupts cartilage formation in endochondral repair. IL-6 facilitates the transition to the reparative phase by stimulating acute-phase protein synthesis and osteoblast maturation, whereas IL-10, predominantly released by M2 macrophages and regulatory T cells, exerts potent anti-inflammatory effects by suppressing TNF-α and IL-1β, attenuating antigen presentation, and

enhancing angiogenesis, ensuring timely resolution of inflammation and progression to bone regeneration (13).

Vascular changes and increased endothelial permeability

Fracture-induced vascular disruption produces local hypoxia and hemorrhage, with the ensuing hematoma serving as both a physical scaffold and a biochemical niche for inflammatory and progenitor cell recruitment. Endothelial cells in adjacent microvessels rapidly upregulate adhesion molecules such as ICAM-1 (intercellular adhesion molecule1) and VCAM-1 (vascular cell adhesion molecule 1), promoting leukocyte adhesion and transmigration into the injured site. During the inflammatory phase, histamine release, bradykinin activation, and cytokine-driven cytoskeletal rearrangements in endothelial cells increase vascular permeability, enabling plasma proteins like fibrinogen to extravasate and form a fibrin-rich provisional matrix that supports nutrient and mediator delivery to the developing callus. Concurrently, angiogenic pathways are activated, with VEGF secreted by macrophages and platelets stimulating capillary sprouting toward hypoxic regions. Timely restoration of perfusion ensures adequate oxygen supply, metabolite clearance, and resolution of inflammation, whereas persistent vascular impairment or insufficient angiogenesis is closely associated with delayed union and nonunion risk, underscoring vascular repair as a critical therapeutic target in fracture management (14).

Soft callus and hard callus phases

During the reparative phase of fracture healing, MSCs originating from the periosteum, bone marrow, and adjacent soft tissues migrate to the injury site, where they differentiate into chondrocytes and osteoblasts under the influence of signaling pathways such as Wnt/β-catenin, bone morphogenetic proteins (BMPs), and TGF-β. Mechanical cues transmitted through the extracellular matrix (ECM) engage pathways including focal adhesion kinase and MAPK, activating transcription factors like Runx2 and osterix to drive osteoblast maturation. As hard callus formation progresses, osteoblasts become embedded within the mineralizing matrix, maturing into osteocytes that maintain bone homeostasis. This tightly orchestrated sequence of progenitor recruitment, biochemical and biomechanical signaling, and spatial organization ensures the replacement of the cartilaginous scaffold with a mineralized structure capable of restoring structural integrity (15).

Production of extracellular matrix and collagen

Formation of a mechanically competent callus depends on the coordinated synthesis and organization of an ECM that serves as both a structural scaffold and a reservoir of biochemical signals. In the soft callus stage, chondrocytes generate a cartilage-rich ECM composed primarily of type II collagen and proteoglycans, providing elasticity, compressive resilience, and a framework for vascular infiltration and mineralization. As healing progresses to the hard callus phase, osteoblasts replace the matrix with type I collagen and non-collagenous proteins, such as osteocalcin, osteopontin, and bone sialoprotein that regulate hydroxyapatite deposition and fiber alignment along mechanical stress lines. This is reinforced by lysyl oxidase-mediated collagen crosslinking and progressive mineral loading, transforming the pliable soft callus into a rigid, load-bearing structure capable of withstanding physiological forces and setting the stage for final bone remodeling (16).

Angiogenesis and development of new vasculature

Effective maturation of both soft and hard callus depends on sufficient vascularization, which delivers oxygen, nutrients, and systemic signaling molecules to support cellular and metabolic activity. Angiogenesis at this stage is driven largely by VEGF released from hypertrophic chondrocytes and osteoblasts, promoting endothelial proliferation, migration, and capillary sprouting into the callus. Matrix metalloproteinase-mediated ECM degradation permits vessel invasion, while pericytes and smooth muscle cells stabilize the forming channels, integrating them into the systemic circulation. Inadequate vascular growth leads to hypoxia, nutrient scarcity, and delayed mineralization, whereas robust vessel formation not only expedites the transition to hard callus but also sustains the subsequent remodeling of woven into lamellar bone (17).

Remodeling phase

The remodeling phase marks the conversion of mechanically immature woven bone into structurally optimized lamellar bone, distinguished by parallel collagen alignment and organized osteonal architecture that greatly enhance strength and resilience. This transformation involves balanced cycles of resorption and deposition, guided by vascular channel formation into existing bone and concentric matrix deposition aligned with prevailing mechanical stresses. The process, which can extend for months or even years after initial fracture union, is strongly influenced by mechanical loading, with regions under greater strain undergoing more extensive adaptation to restore full biomechanical competence and minimize refracture risk (18).

Coordinated activity of osteoclasts and osteoblasts

Bone remodeling is driven by the coordinated actions of osteoclasts and osteoblasts within specialized remodeling units, where osteoclast-mediated matrix resorption is precisely followed by osteoblast-driven deposition and mineralization of lamellar bone. This coupling, regulated by signaling pathways such as RANKL–OPG (receptor activator of nuclear factor kappa-B ligand- osteoprotegerin) and paracrine mediators like TGF-β, preserves skeletal homeostasis and ensures structural optimization after

fracture repair. Any disruption in this balance, whether due to metabolic disorders, hormonal alterations, or insufficient mechanical loading can impair remodeling efficiency, with overactive resorption weakening bone and inadequate turnover leaving inferior tissue uncorrected. Targeted modulation of these cellular interactions offers therapeutic potential to enhance the quality and strength of the healed bone (19).

Findings related to the pathogenesis of nonunion

Dysregulation of the inflammatory response is a key contributor to fracture nonunion, with both under- and overactivation impairing healing. Inadequate inflammation, as seen in immunosuppression, aging, malnutrition, and chronic disease, limits mesenchymal progenitor recruitment and angiogenesis, preventing robust callus formation. Conversely, excessive or prolonged inflammation, often associated with severe soft-tissue injury or systemic inflammatory states, causes oxidative damage, ECM breakdown, and reduced chondrocyte and osteoblast viability. Maintaining a balanced immune response is essential for timely progression to the reparative phase, and early inflammatory profiling may aid in identifying patients at risk and tailoring immunomodulatory therapies to optimize outcomes (20) (Figure 1).

Persistent pro-inflammatory cytokine expression and detrimental effects

Persistent elevation of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6 can drive certain nonunion phenotypes by maintaining a catabolic microenvironment that promotes osteoclastogenesis while suppressing osteoblast differentiation and matrix synthesis. This sustained inflammatory state destabilizes vasculature, impairs angiogenesis, and induces oxidative endothelial injury, leading to hypoxia and further osteocyte loss. The resulting cycle of cellular damage and cytokine release perpetuates failed mineralization. Emerging targeted therapies, including cytokine blockade and modulation of macrophage phenotype, aim to suppress harmful inflammation locally while preserving the immune functions essential for early fracture repair (21).

Cellular dysfunctions

A significant reduction in osteoblast and stromal cell populations within the fracture milieu is a well-recognized barrier to successful bone regeneration. Osteoblasts, derived from mesenchymal progenitors, are indispensable for the synthesis of new ECM and the initiation of mineralization. Conditions such as chronic diseases, systemic glucocorticoid exposure, smoking, and metabolic disorders can diminish both the number and functional competence of these cells. In such settings, the anabolic drive essential for callus maturation is sharply curtailed, hampering mechanical consolidation (22).

Imbalance in M1/M2 macrophage polarization

A marked reduction in osteoblast and stromal cell populations is a major impediment to fracture healing, as these cells are essential for ECM synthesis, mineralization, and structural maturation of the callus. Factors such as chronic illness, glucocorticoid therapy, smoking, and metabolic disorders can diminish their numbers and compromise functional capacity, resulting in decreased collagen and osteocalcin production, poor hydroxyapatite deposition, and inadequate paracrine support for osteogenesis. This cellular deficit disrupts the balance between resorption and formation, predisposing to fibrous or cartilaginous nonunion with persistent instability. Emerging interventions aim to enhance stromal cell viability, stimulate osteoprogenitor recruitment, or deliver exogenous osteoblasts to restore regenerative potential (23).

Microvascular abnormalities

Adequate angiogenesis is essential for fracture repair, providing the oxygen, nutrients, and molecular signals necessary for osteogenesis and chondrogenesis. Impaired vessel formation, resulting from reduced endothelial proliferation, migration defects, or dysregulated factors such as VEGF and angiopoietins limits microvascular

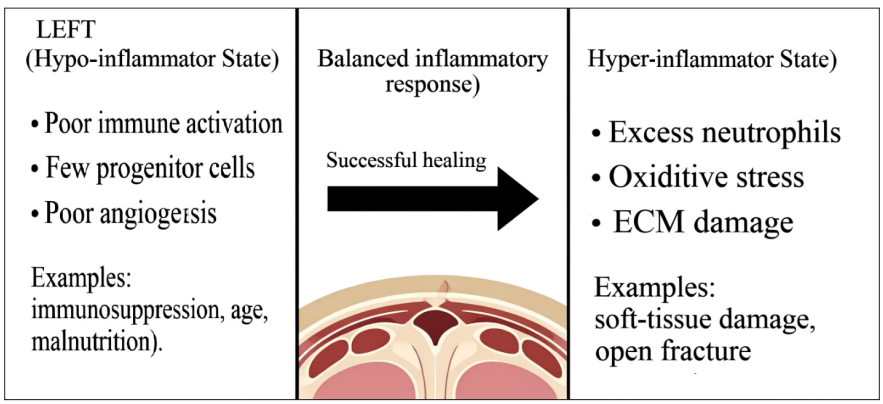

Figure 1. Hypo- and hyper-inflammatory pathways disrupting bone healing.

density, causing nutrient deprivation, ECM insufficiency, and premature cell death. Poor vascular invasion of the cartilaginous callus delays its replacement with mineralized bone, predisposing to hypovascular, fibrotic nonunion. Therapeutic strategies including growth factor delivery, VEGF-targeted gene therapy, and vascularized grafts aim to enhance vessel density, maturity, and stability to restore metabolic support throughout healing (24).

Local ischemia

Local ischemia is a major impediment to fracture healing, as sustained hypoxia and nutrient deprivation compromise the survival and function of osteoblasts, chondrocytes, and endothelial cells. Often arising from traumatic vascular injury, excessive periosteal stripping, or postoperative complications, prolonged ischemia fosters acidosis, oxidative stress, and impaired clearance of inflammatory mediators, creating a persistent hostile environment that stalls callus maturation and mineralization. Management strategies emphasize preservation or restoration of vascular supply through careful surgical technique, timely soft tissue repair, and microsurgical revascularization, supplemented by adjunctive measures such as hyperbaric oxygen, vasodilatory agents, or mechanical stimulation to enhance local perfusion and support bone regeneration (25).

Systemic risk factors

Fracture healing is adversely influenced by systemic and lifestyle factors such as diabetes, smoking, and certain medications. Chronic hyperglycemia in diabetes impairs osteoblast function, alters ECM quality through glycation, and limits tissue perfusion via microangiopathy, collectively prolonging repair and increasing nonunion risk. Smoking compounds vascular compromise by reducing oxygen delivery, impairing endothelial migration, and shifting bone turnover toward resorption, with epidemiological evidence linking it to delayed consolidation. Pharmacologic agents including NSAIDs and corticosteroids further disrupt repair by blunting inflammatory signaling, inhibiting osteoblastogenesis, promoting osteocyte apoptosis, and suppressing angiogenesis, highlighting the importance of addressing modifiable risks and optimizing medical therapy in fracture patients (26).

Radiological and histological patterns in nonunion

Radiographically, nonunions are classified as atrophic or hypertrophic, each reflecting distinct biological and mechanical deficits. Atrophic nonunions exhibit little or no callus formation with absent cortical bridging, indicating poor osteogenic activity often linked to vascular compromise, infection, or systemic metabolic disorders. In contrast, hypertrophic nonunions display abundant callus with characteristic "elephant's foot" morphology, reflecting preserved biology but inadequate mechanical stability due to insufficient fixation or excessive micromotion. Differentiating these subtypes is essential, as atrophic lesions require biological stimulation, whereas hypertrophic cases benefit primarily from mechanical reinforcement (27).

Microscopic and histopathological findings

Histologically, atrophic nonunions are marked by fibrous tissue filling the fracture gap, sparse or absent osteoblast activity, reduced marrow cellularity, and poorly organized, minimally mineralized collagen, sometimes with degenerating chondrocytes. Hypertrophic nonunions, in contrast, contain abundant fibrocartilage, active osteoid production, and numerous maturing chondrocytes, yet mineralization remains incomplete and resorption lacunae are common from mechanical strain. Irregular but present vascular channels and occasional inflammatory infiltration further reflect the disrupted repair environment. These microscopic features complement imaging, offering critical guidance for tailored therapeutic strategies (28).

Biomarkers and molecular findings in healing and nonunion

Elevations in systemic inflammatory markers such as C-reactive protein, erythrocyte sedimentation rate, and IL-6 are common in fracture nonunion, reflecting either sterile inflammation or occult infection. C-reactive protein, a hepatic acute phase reactant, is sensitive to cytokine-driven changes at the fracture site, while ESR offers a broader but less specific measure of inflammatory load. IL-6 not only amplifies inflammation but also promotes osteoclastogenesis via the RANK/RANKL pathway, tipping the balance toward bone resorption and impairing repair. Integrating these biomarkers into clinical evaluation aids in identifying biologically unfavorable healing conditions and guiding targeted therapeutic strategies (29).

Bone formation markers (ALP, osteocalcin and P1NP)

Bone formation markers such as bone-specific alkaline phosphatase, osteocalcin, and procollagen type 1 N-propeptide (P1NP) offer valuable insights into osteoblast activity and ECM synthesis. Reduced levels of these markers in nonunion indicate impaired osteogenesis, with low alkaline phosphatase reflecting inadequate mineral deposition, diminished osteocalcin suggesting defective matrix maturation, and decreased P1NP pointing to reduced type I collagen synthesis. Serial monitoring of these biomarkers, alongside imaging, helps differentiate biologically inactive from active nonunion, guiding the need for targeted biologic augmentation when anabolic capacity is compromised (30).

Vascularization markers

Gene expression studies in nonunion tissue reveal suppression of key osteogenic and matrix genes alongside heightened expression of inflammatory mediators and matrix-degrading enzymes, creating a microenvironment

unfavorable for repair. Proteomic analyses further demonstrate disrupted networks governing ECM integrity, metabolic activity, and growth factor signaling. Integrating transcriptomic and proteomic insights offers a comprehensive view of the molecular deficits driving nonunion, paving the way for biomarker discovery and targeted therapeutic interventions (31).

Immunohistochemistry results

Immunohistochemistry in nonunion tissue reveals localized deficits in repair-related proteins, with reduced osteocalcin, ALP, and P1NP indicating diminished osteoblast activity and elevated matrix metalloproteinases signifying active matrix degradation. Atrophic lesions typically show minimal angiogenic and osteogenic staining, consistent with poor vascularity and cellularity, whereas hypertrophic lesions often present abundant but disorganized VEGF-positive vessels linked to mechanical instability. These staining profiles align with molecular assays and offer clinically relevant insights for tailoring therapeutic approaches to specific nonunion patterns (32).

Biological strategies in the treatment of nonunion

Bone morphogenetic proteins, particularly BMP-2 and BMP-7, are key osteoinductive factors that stimulate MSC differentiation into osteoblasts via Smad-mediated gene activation, enhancing matrix synthesis and mineralization. Local administration in nonunion has improved healing outcomes, especially with recombinant BMP-2 in combination with stable fixation, though variability in response, dosing optimization, and delivery methods remain challenges. Potential complications such as heterotopic ossification, inflammatory reactions, and high cost underscore the need for careful patient selection and the development of safer, more cost-effective BMP-based therapies (33).

Platelet-derived growth factor and TGF-β

Platelet-derived growth factor promotes angiogenesis, fibroblast proliferation, and MSC recruitment, supporting the transition from inflammation to tissue repair, while TGF-β regulates chondrocyte activity, ECM production, and osteoblast differentiation, facilitating endochondral ossification. Both have therapeutic potential in nonunion, but excessive or imprecise dosing risks fibrosis and disproportionate matrix deposition without proper mineralization, highlighting the need for controlled-release delivery systems to optimize their local concentration and timing (34).

Cell-based therapies

Mesenchymal stem cell therapy offers a versatile approach to nonunion repair, with bone marrow-, adipose-, and amniotic-derived sources capable of differentiating into osteoblasts, chondrocytes, and endothelial cells while secreting regenerative cytokines. Delivery methods include direct injection or scaffold-based implantation, often enhanced by combining with osteoinductive agents like BMPs. Adipose-derived MSCs provide higher yields and strong immunomodulatory effects, whereas autologous use minimizes immune rejection. Key challenges remain in maintaining cell viability, standardizing preparation, and avoiding unwanted lineage commitment, though emerging allogeneic "off-the-shelf" products promise improved accessibility (35).

Endothelial progenitor cells

Endothelial progenitor cells facilitate fracture healing by homing to hypoxic sites, differentiating into endothelial cells, and releasing angiogenic factors like VEGF that indirectly promote osteogenesis. Experimental models show EPC transplantation increases callus vascularity, while early clinical efforts focus on optimizing delivery, safety, and bone marrow mobilization to enhance endogenous repair (36).

Tissue engineering and scaffolds

Calcium phosphate biomaterials, particularly hydroxyapatite, offer osteoconductive, biocompatible scaffolds with tunable porosity to support vascularization and bone ingrowth, while graphene-based components add mechanical strength, electrical conductivity, and osteoinductive potential. Composite designs combining these materials can provide durable structural support, controlled biodegradation, and enhanced recruitment of regenerative cells (35).

Hybrid and bioactive scaffolds

Hybrid scaffolds combine ceramics, polymers, and bioactive coatings to provide mechanical stability while delivering biological cues, often incorporating growth factors or cells for targeted regeneration. Bioactive surface modifications enhance progenitor cell attachment and differentiation, and advances in 3D printing enable patient-specific designs with optimized architecture and porosity, offering tailored solutions for complex or segmental nonunions (37).

Targeted anti-inflammatory agents

Tumor necrosis factor-α plays a dual role in fracture healing, supporting early inflammation but impairing regeneration when chronically elevated. Targeted TNF-α inhibition can reduce osteoclast activity and enhance osteoblast survival, with optimal benefit dependent on timing. Experimental strategies in nonunion focus on localized delivery via sustained-release hydrogels or coated implants to limit systemic immunosuppression (38).

Modulators of macrophage activation

Shifting macrophages from a pro-inflammatory M1 to a pro-reparative M2 phenotype enhances bone healing by dampening harmful inflammation and promoting

angiogenesis and matrix formation. Local delivery of cytokines like IL-4 and IL-13, including via biomaterial scaffolds, has shown preclinical success, positioning targeted immune modulation as a key strategy in advanced nonunion therapies (39).

Findings from animal and in-vitro models

Animal models are essential for studying fracture repair and nonunion, with rodents offering cost-effective, genetically modifiable platforms for mechanistic research, rabbits providing intermediate healing rates and clinically relevant cortical bone for biomaterials testing, and canines delivering high translational value for load-bearing fracture studies. Each species presents unique advantages and limitations in healing kinetics, bone biology, and applicability, making careful model selection critical to align experimental design with research objectives (40).

Similarities and differences compared to human healing

Animal and human fracture healing share core biological pathways including inflammation, callus formation, ossification, and remodeling mediated by conserved cell types and growth factors, yet differ markedly in kinetics, biomechanics, and anatomical features. Rodents heal rapidly with thin cortices and high turnover, while humans exhibit slower consolidation influenced by complex loading patterns, periosteal structure, and systemic health factors. These differences mean that while animal models are invaluable for identifying biological targets and testing therapies, their translation to clinical nonunion requires caution, particularly for interventions involving systemic inflammation, angiogenesis, or mechanical stability (41).

Strengths and limitations of preclinical evidence

Preclinical fracture models provide valuable insight into the cellular and molecular basis of bone repair and serve as critical platforms for testing new therapies under tightly controlled conditions, enabling precise variable manipulation and efficient screening of biomaterials, biologics, and mechanical strategies. While these models enhance mechanistic understanding and reduce early clinical trial risks, their reliance on young, healthy animals, simplified fracture patterns, and absence of comorbidities limits direct clinical translation. Differences in biomechanics, immune responses, and follow-up duration further challenge applicability, underscoring the need to interpret findings within these constraints and bridge them with carefully designed clinical studies (42).

Comparative findings between conventional and biological treatments

Conventional fracture nonunion treatments focus on restoring mechanical stability through revision fixation, implants, or bone grafting, while relying on the patient's natural healing capacity, which may be insufficient in biologically compromised cases. Biological approaches, including growth factors, stem cell therapies, and scaffold-based delivery systems, directly target deficits in osteogenesis, angiogenesis, or inflammation to accelerate repair. Emerging evidence supports combined strategies that pair stable fixation with biological augmentation for improved union rates and functional outcomes, though their broader use is limited by cost, regulatory challenges, and variable patient response, underscoring the need for personalized, biomarker-guided application (43).

Discussion

Fracture healing depends on coordinated inflammation, angiogenesis, and osteogenesis, with disruptions in any stage predisposing to nonunion (44). Insights from animal and in vitro models have clarified that early vascular disruption, prolonged pro-inflammatory cytokine activity, and insufficient osteoprogenitor recruitment are recurrent mechanisms underlying impaired union. Rodent models permit precise genetic and molecular interrogation, whereas rabbit and canine systems more closely replicate human cortical structure, mechanical demands, and implant behavior. While the fundamental stages and key molecular mediators, such as growth factors, inflammatory cytokines, and osteogenic cells are conserved between species, differences in healing kinetics, biomechanics, marrow cell density, and comorbidity profiles pose significant challenges for direct translation to clinical settings. These models remain particularly valuable for elucidating causal mechanisms, evaluating novel biomaterials, and piloting orthobiologic concepts, though the controlled experimental conditions—often involving young, healthy subjects and standardized fracture patterns rarely mirror the complex clinical reality of high-energy injuries, systemic disease, or advanced age (45).

Clinically, mechanical strategies like revision fixation, intramedullary nailing, and autologous bone grafting form the cornerstone of nonunion management, offering mechanical stability and osteoconductive scaffolding but relying on intact host regenerative capacity (43). In biologically compromised environments—such as atrophic nonunion—emerging biologic approaches aim to supply targeted osteoinductive, angiogenic, or anti-inflammatory cues using recombinant BMPs, platelet-derived factors, stem cells, gene therapies, and scaffold-based delivery systems (46). Preclinical and early clinical evidence increasingly supports integrated treatment paradigms that combine biomechanical optimization with biologic augmentation, fostering a microenvironment conducive to angiogenesis, osteoblast recruitment, and resolution of chronic inflammation. Despite this promise, clinical integration faces barriers including cost, regulatory oversight, variable patient responsiveness, and incomplete understanding of optimal dosing and delivery. The heterogeneity of nonunion pathophysiology demands mechanistic stratification—distinguishing hypertrophic forms driven by instability from atrophic lesions due

to biological insufficiency—and the use of predictive biomarkers to guide personalized intervention. Ultimately, bridging preclinical advances with rigorously designed clinical trials will be essential to improving union rates and functional recovery in this challenging complication (37).

Conclusion

In summary, advancing fracture nonunion management requires an integrated strategy that unites mechanical stability with targeted biological enhancement. Insights from robust preclinical models, combined with translational clinical evidence, underscore the importance of tailoring interventions to the underlying pathophysiology. By aligning fixation techniques with patient-specific biological augmentation, future approaches hold promise for improving healing rates, restoring function, and reducing the long-term burden of this challenging orthopedic complication.

Authors' Contribution

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Investigation: Sina Najafi.
Methodology: Asghar Elmi and Ahmad Behroz Rasikh.
Project administration: Ahmad Behroz Rasikh.
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Software: Sina Najafi.
Supervision: Ahmad Behroz Rasikh.
Validation: Ahmad Behroz Rasikh and Asghar Elmi.
Visualization: Sina Najafi and Asghar Elmi.
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Conflicts of interest

The authors declare that they have no competing interests

Ethical issues

Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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