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REVIEW

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The bone microenvironment: new insights into the role of stem cells and cell communication in bone regeneration

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Abstract

Mesenchymal stem cells (MSCs) play a crucial role in bone formation and remodeling. Intrinsic genetic factors and extrinsic environmental cues regulate their differentiation into osteoblasts. Within the bone microenvironment, a complex network of biochemical and biomechanical signals orchestrates bone homeostasis and regeneration. In addition, the crosstalk among MSCs, immune cells, and neighboring cells—mediated by extracellular vesicles and non-coding RNAs (such as circular RNAs and micro RNAs) —profoundly influences osteogenic differentiation and bone remodeling. Recent studies have explored specific signaling pathways that contribute to effective bone regeneration, highlighting the potential of manipulating the bone microenvironment to enhance MSC functionality. The integration of advanced biomaterials, gene editing techniques, and controlled delivery systems is paving the way for more targeted and efficient regenerative therapies. Furthermore, artificial intelligence could improve bone tissue engineering, optimize biomaterial design, and enable personalized treatment strategies. This review explores the latest advancements in bone regeneration, emphasizing the intricate interplay among stem cells, immune cells, and signaling molecules. By providing a comprehensive overview of these mechanisms and their clinical implications, we aim to shed light on future research directions in this rapidly evolving field.

Keywords Bone microenvironment, Mesenchymal stem cells, Osteogenesis, Differentiation, Biomaterials

Introduction

Osteogenesis is the complex process of bone formation, which includes the production of the bone matrix, mineralization, and bone maturation. Osteogenesis requires osteoblastogenesis, which is the more specific process of mesenchymal stem cell (MSC) differentiation into osteoblasts, the cells responsible for synthesizing the bone matrix and for its mineralization. Thus the objective of the present review is to update our current knowledge in bone regeneration, considering its regulation by cell signaling pathways, the impact of the bone microenvironment, and the promise held by innovative technologies currently under development.

To identify relevant research findings focusing on Mesenchymal Stem Cells (MSCs) and their interactions within the bone microenvironment, we performed an extensive online search using keywords such as 'MSCs,' 'bone regeneration,' 'cellular interactions,' and 'bone microenvironment.' This search covered peer-reviewed journals and databases, including PubMed, Scopus, Google Scholar, and Web of Science. This approach aimed to select a diverse spectrum of experimental and clinical studies, enabling us to summarize the latest

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advancements and emerging trends in the field of bone regeneration. In our analysis, we evaluated the collected data to identify gaps in current research and propose future directions. This approach involved assessing the methodologies, study designs, and outcomes reported in the literature, in order to provide a comprehensive overview of our current knowledge and highlight novel areas for further investigation.

The bone microenvironment and its role in bone regeneration

Cellular communication within the bone microenvironment

The bone microenvironment is crucial for maintaining bone health and supporting its regeneration after injury. This dynamic network, consisting of cells, extracellular matrix (ECM), and signalling molecules, forms a niche that influences the metabolism of resident cells, especially MSCs and osteoblasts. Osteoblasts (derived from MSCs) are the primary bone builders. They act by synthesizing and depositing bone matrix in response to hormonal and mechanical signals during bone growth and repair [1]. During the calcification process, osteoblasts become osteocytes. Osteocytes are quiescent cells embedded into the bone matrix, which regulate mineral homeostasis and respond to mechanical signals (including mechanosensory ones) that influence the adaptive response of the bone [2]. Osteoclasts, which are derived from fused monocytes/macrophages, are responsible for bone resorption, a crucial process for its remodelling and repair, through the action of digestive enzymes [3]. The bone microenvironment also includes endothelial and immune cells. Endothelial cells form the vascular network within the bone, which is essential for nutrient supply and immune cell transport [4]. Endothelial cells help to regulate the blood flow and inflammatory response, working in conjunction with immune cells (such as macrophages, T cells, and B cells), which modulate inflammation and maintain bone homeostasis [4–6]. These different cell populations form interaction networks through complex molecular and mechanical signals, to maintain bone integrity, respond to damage or mechanical stress, and regulate processes such as bone mineralization and local immune response. During tissue repair, cell adhesion, migration, and proliferation, the bone matrix provides a scaffold that supports cellular functions [7, 8]. The structural integrity of the bone matrix not only ensures mechanical stability but also drives the spatial organization of cells involved in the bone healing process [9, 10]. The bone microenvironment is rich in growth factors, cytokines, and ECM proteins that regulate cellular activities, thereby ensuring bone homeostasis [8, 11]. This homeostatic equilibrium involves a delicate interplay between bone-forming osteoblasts and bone-resorbing osteoclasts, which is orchestrated by paracrine signalling and systemic factors [12]. Growth factors, such as bone morphogenetic proteins (BMPs) and transforming growth factor-beta (TGF-β), are pivotal in promoting the osteogenic differentiation of MSCs into osteoblasts. These factors also regulate osteoclast activity, which is essential for bone resorption and remodelling during the bone healing process [11]. Moreover, the bone microenvironment regulates the intricate cellular crosstalk among osteoblasts, osteoclasts, and immune cells. [13–15].

Osteoblastogenesis is regulated by the equilibrium between intrinsic and extrinsic factors, which emphasizes the importance of the bone microenvironment in promoting their interaction. The bone microenvironment orchestrates intricate cellular interactions and signaling pathways that are crucial for maintaining bone homeostasis and facilitating its effective regeneration [9, 13, 16, 17]. This communication involves biomechanical and biochemical signals, such as mechanical stimuli and interactions among various cell types within the bone tissue (Fig. 1).

The magnifying lens represented in Fig. 1 highlights the signaling pathways that control the differentiation of MSCs into osteoblasts. These signalling pathways are pivotal in regulating bone regeneration and remodelling. Several recent publications demonstrate the integration of these signaling pathways, which include BMP, Wnt, Notch, Hedgehog, and Fibroblast Growth Factors (FGFs), in the differentiation of MSCs into osteoblasts [18]. The molecular mechanisms associated with these signaling pathways are detailed in Fig. 2.

The Wnt signalling pathway (Fig. 2, center) regulates MSC proliferation, differentiation into osteoblasts, and bone formation [18]. In particular, Wnts, which comprise different secreted molecules (nineteen members have been described in mouse and human genomes), transmit signals through various pathways [19, 20]. The canonical Wnt/β-catenin pathway is the most studied, being crucial for embryonic development and tissue stability [21, 22]. In this pathway, the stability of β-catenin is pivotal and regulated by phosphorylation and degradation when Wnt ligands are absent. Binding of Wnt ligands to Frizzleds (Fz) and LDL Receptor Related Protein 5/6 (LRP5/6) coreceptors disrupts the β-catenin degradation complex, thereby allowing β-catenin to accumulate and enter the nucleus [23]. Mutations affecting LRP5 can lead to conditions like osteoporosis-pseudoglioma or an increased bone mass [24]. As we reported above, BMPs (Fig. 2, center) are potent inducers of osteogenesis and bone formation. They stimulate MSC differentiation

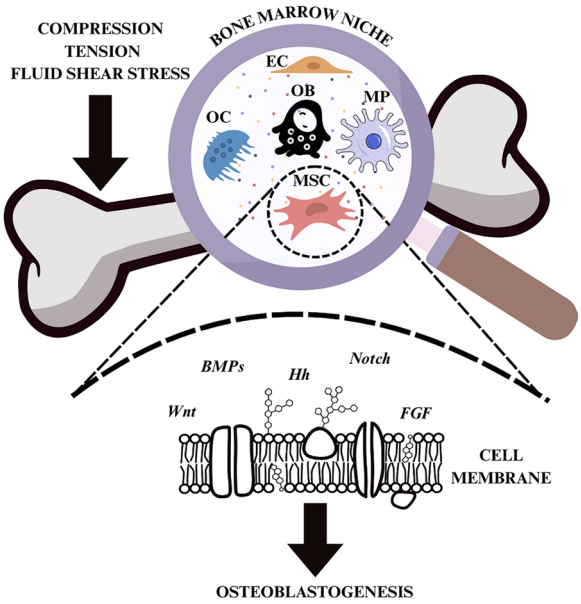

Fig. 1 Extrinsic and intrinsic factors that regulate osteoblastogenesis. Biomechanical signals, such as mechanical stress, compression, and fluid shear stress, are crucial in regulating osteoblastogenesis. Bone tissue is constantly subjected to mechanical forces, and cells within the bone microenvironment are sensitive to these stimuli. For example, osteoblasts (OB) and osteoclasts (OC) are responsive to mechanical loading, which influences their activity. Compression forces can promote osteoblast differentiation, while fluid shear stress (the force exerted by the flow of interstitial fluid in response to mechanical loading) affects both osteoblast and osteoclast function. These biomechanical signals are crucial in adapting bone structure and function to the mechanical demands placed on it. In addition, the cellular interactions during osteoblastogenesis are intricate and multi-directional. Endothelial cells (ECs), which line blood vessels, play a key role in regulating the vascular environment and can influence osteoblast and osteoclast activity. Osteoclasts, which are the cells responsible for bone resorption, interact with osteoblasts in a balanced manner to ensure that bone homeostasis is maintained. Macrophages (MPs) play a vital role in the immune response within bone tissue, and their interactions modulate osteoblastogenesis. Mesenchymal stem cells (MSCs) differentiate into osteoblasts, through cell signalling networks involving the Wnt, bone morphogenic proteins (BMPs), Hedgehog (Hh), Notch, and fibroblast growth factors (FGF) pathways. The image was created using Canva: https://www.canva.com/

into osteoblasts and promote matrix mineralisation during bone repair and regeneration. In addition, Hedgehog (Hh) signalling (Fig. 2, right side), involving Smoothened (Smo) and Patched1 (PTCH1) receptors, activates downstream genes crucial for development and tissue maintenance [25–27]. PTCH1 serves as a key regulator, by inhibiting Smo in the absence of Hh ligand [27]. Mutations in PTCH1 can dysregulate Hh signaling, contributing to skeletal abnormalities and cancer predisposition in conditions including Gorlin syndrome [18]. Cell fate decisions and bone tissue differentiation are also influenced by Notch signalling (Fig. 2, center). This pathway regulates osteoblast and osteoclast precursor differentiation, contributing to bone remodeling and maintenance. Similar to BMP and Wnt signaling, RUNX2 function is also affected by Notch signaling. The transcriptional activity of RUNX2 is directly countered by the protein encoded by the Notch target gene Hey1 [28]. Furthermore, the Hippo signalling pathway, a serine/threonine kinase cascade, plays a crucial role in regulating osteoblastogenesis (Fig. 2, left side). Through its primary transcriptional co-factors, Yes-associated protein (YAP) and transcriptional co-activator with PDZ-binding motif (TAZ), the Hippo pathway affects the lineage commitment and regulation of various bone-related cell populations [29]. The YAP/TAZ protein pair has been demonstrated to enhance the lineage commitment of osteoblasts instead of promoting adipogenesis. Shear stress and ECM stiffness influence the extent of nuclear translocation of YAP/TAZ in MSCs [30, 31] (Fig. 2, left). In particular, on a stiff substrate, there is increased clustering of integrins and formation of focal adhesions, which enhances the polymerization of F-actin and the generation of stress fibers. The increased clustering of integrins induces torsional forces within the stress fibers, promoting the translocation of YAP/TAZ from the cytosol to the nucleus. The nuclear translocation of YAP/TAZ stimulates osteogenesis by upregulating RUNX2 and downregulating the adipogenic transcription factor peroxisome proliferator-activated receptor gamma (PPARγ). Conversely, a soft substrate does not support the formation of focal adhesions and stress fibers, causing the cell to become more rounded and with a smaller spreading area. This alteration leads to the sequestration of YAP/TAZ in the cytosol, which drives adipogenesis by upregulating PPARγ and downregulating RUNX2 [32].

Additionally, mechanical stimuli exerted on bone tissue, such as weight-bearing activities and muscle contractions, play a crucial role in bone adaptation and regeneration [33]. The stiffness, density, and architecture of the bone matrix influence cell behavior and differentiation [34, 35]. Mechanical cues sensed by osteoblasts and osteocytes regulate bone formation and remodeling processes.

Bioactive molecules such as interleukins, TGF-β, and growth factors mediate the communication between bone cells and immune cells, influencing various processes [36, 37]. Pro-inflammatory cytokines such as interleukin-1 (IL- 1) and interleukin- 6 (IL- 6) regulate osteoclast activity and bone resorption [38, 39]. Conversely, anti-inflammatory cytokines including interleukin- 10 (IL- 10) can suppress osteoclastogenesis and promote osteoblast function [40]. TGF-β regulates osteoblast proliferation, ECM production, and bone matrix deposition [41]. It also

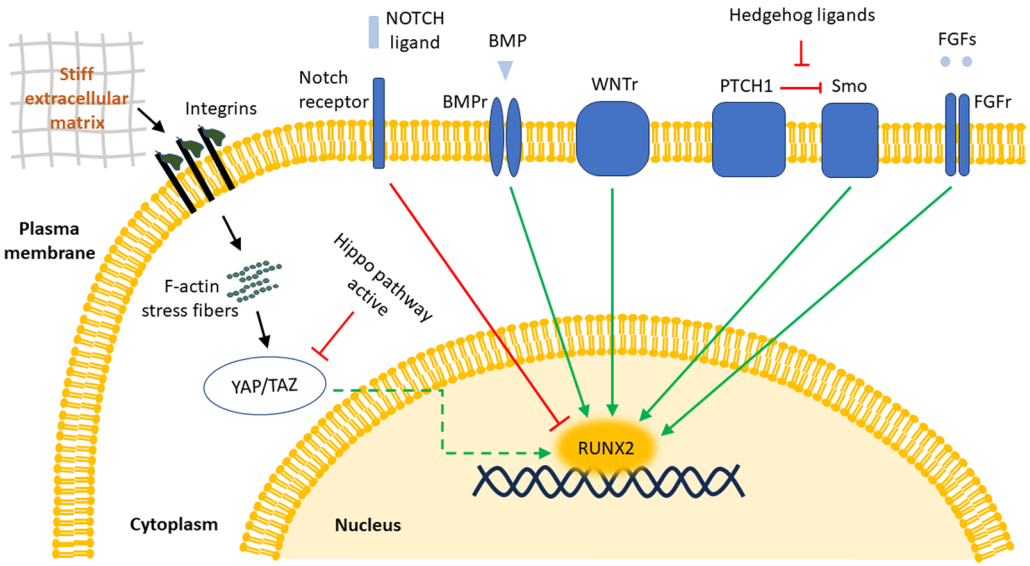

Fig. 2 Signalling pathways that regulate osteoblastogenesis. Osteoblastogenesis is tightly controlled by numerous signaling pathways that regulate the activity of key osteogenic transcription factors, such as Runt-related transcription factor 2 (RUNX2), which are essential for bone regeneration and remodeling. Signaling pathways, including Hippo, Notch, Bone Morphogenetic Protein (BMP), Wnt, Hedgehog, and Fibroblast Growth Factors (FGFs), play a distinct role in regulating osteogenesis, by either promoting or inhibiting the expression of the gene coding for RUNX2. The canonical Wnt/β-catenin pathway is one of the most well-known pathways that positively regulates osteogenesis. Upon binding of Wnt ligands to their receptors (WNTr), β-catenin is stabilized, preventing its degradation. This stabilization allows β-catenin to accumulate in the nucleus, where it promotes the transcription of genes involved in osteoblast differentiation, including RUNX2. Identical outcomes occur when BMP or FGFs bind to their receptors (BMPr and FGFr, respectively), activating the transcription of the messenger RNA coding for RUNX2. Similarly, Hedgehog signaling, modulated by the Smoothened (Smo) and Patched1 (PTCH1) receptors, also supports RUNX2 activity. Hedgehog signaling is crucial not only for osteoblast differentiation but also for the regulation of bone development and tissue maintenance. It interacts with various cellular processes to ensure that osteoblast differentiation occurs properly during skeletal development. On the other hand, Notch signaling exerts a negative regulatory effect on osteogenesis. Upon ligand activation, the Notch receptor interacts with the transcription factor Hey1, which inhibits RUNX2 activity and thus affects both cell differentiation and bone remodeling. This impact of this pathway in bone biology underscores its importance in regulating the balance between osteoblast and osteoclast activity, ensuring proper bone turnover. Another pathway that influences osteoblast differentiation is the Hippo signaling pathway. This pathway regulates cell proliferation and survival. It can negatively impact osteolastogenesis by inhibiting the Yes-associated protein (YAP) and the transcriptional co-activator with PDZ-binding motif (TAZ). When the Hippo pathway is activated, the YAP/TAZ protein pair remains inactive in the cytoplasm, thus preventing osteoblast differentiation. In contrast, when the extracellular matrix (ECM) stiffness increases, integrins cluster and induce the formation of F-actin stress fibers, leading to the translocation of YAP/TAZ into the nucleus (represented as a green dashed line), where they activate the expression of genes associated with osteoblast differentiation, such as RUNX2. The mechanical properties of the ECM (particularly its stiffness) play a crucial role in regulating YAP/TAZ activity. In contrast with a stiff ECM, a softer ECM inhibits the activation of YAP/TAZ, thus preventing osteoblast differentiation. This mechanosensitive regulation highlights the importance of biomechanical forces in the fine-tuning of osteoblastogenesis, a critical step in bone remodeling and regeneration

regulates immune cell function and contributes to tissue repair processes [42, 43]. Cell surface receptors including integrins facilitate cell–cell and cell-ECM interactions within the bone microenvironment [44]. Integrins also have important functions in osteoblast adhesion, migration, and differentiation in response to mechanical and biochemical stimulations [45, 46].

It is well known that osteoblast-osteoclast Interactions are important in the maintenance of bone homeostasis through a coordinated series of activities [46, 47]. Osteoblasts produce osteoid, a collagen-rich matrix that mineralizes to form bone tissue, while osteoclasts, derived from monocyte/macrophage lineage, resorb bone tissue through acidification and enzymatic degradation [48]. Coupling factors including the Receptor Activator of Nuclear Factor Kappa-B Ligand (RANKL) and osteoprotegerin (OPG) regulate the equilibrium between osteoblast-mediated bone formation and osteoclast-mediated bone resorption. Importantly, newly identified molecules that regulate osteoclast activity, collectively known as 'clastokines' (Table 1), have been shown to play a crucial role in influencing bone resorption and remodeling processes [48]. Among the clastokines involved in bone homeostasis, the following are particularly noteworthy for stimulating bone formation: complement component 3a (C3a), Wnt

Table 1 Clastokines involved in bone remodeling

Clastokine	Function	References
Complement component 3a (C3a)	Stimulates bone formation	[48, 57]
Wnt Family Member 5 A (WNT5 A)	Stimulates bone formation	[58]
Slit Guidance Ligand 3 (SLIT3)	Stimulates bone formation	[59]
Cardiotrophin- 1 (CT- 1)	Stimulates bone formation	[49]
Leukemia inhibitory factor (LIF)	Stimulates bone formation	[60, 61]
Tartrate-resistant acid phosphatase (TRAcP)	Stimulates bone formation	[49–51]
Sphingosine 1-phosphate (S1P)	Stimulates bone formation	[49, 50, 52–54]
Bone morphogenetic protein 6 (BMP6)	Stimulates bone formation	[49]
Int/wingless 10b (Wnt10b)	Stimulates bone formation	[49]
Hepatocyte growth factor (HGF)	Stimulates bone formation	[48]
Collagen triple helix repeat containing 1 (CTHRC1)	Stimulates bone formation	[48]
Platelet-derived growth factor BB (PDGF BB)	Inhibits bone formation	[56]
Sclerostin (SOST)	Inhibits bone formation	[63]
Semaphorin 4D (SEMA4D)	Inhibits bone formation	[50, 65]

family member 5 A (WNT5 A), slit guidance ligand 3 (SLIT3), cardiotrophin- 1 (CT- 1), leukemia inhibitory factor (LIF), tartrate-resistant acid phosphatase (TRAcP), sphingosine 1-phosphate (S1P), bone morphogenetic protein 6 (BMP6), int/wingless 10b (Wnt10b), hepatocyte growth factor (HGF), and collagen triple helix repeat containing 1 (CTHRC1) [48–61]. Conversely, platelet-derived growth factor BB (PDGF BB) and sclerostin (SOST) are relevant for inhibiting bone formation [62–64]. The roles of clastokines are currently under investigation, both in vitro and in vivo, and it is widely acknowledged that these molecules act as coupling factors [48].

Recent research has demonstrated the role of extracellular vesicles (EVs) and non-coding RNAs, such as circular RNAs (circRNAs) and microRNAs (miRNAs), in orchestrating cellular communication within the bone microenvironment [66–71]. EVs, which include exosomes and microvesicles, are recognized as crucial mediators of intercellular communication, by transferring bioactive molecules such as proteins, lipids, and RNAs between cells. Within the bone microenvironment, EVs derived from various cell types, including MSCs and immune cells, play a significant role in modulating osteogenic differentiation, bone remodeling processes, and immune responses [72]. Similarly, circRNAs and miRNAs have emerged as important regulators of gene expression and signaling pathways relevant to bone homeostasis and regeneration [73]. Their ability to fine-tune gene expression through post-transcriptional mechanisms makes circRNAs and miRNAs pivotal factors in influencing MSC fate decisions, osteoblast differentiation, and osteoclast activity [74]. In particular, circRNAs play crucial roles in bone homeostasis by regulating bone metabolism and remodeling processes. For example, circ_0006859 inhibits osteogenesis and promotes adipogenesis by targeting the signalling pathway regulated by miR- 431 - 5p/Rho-associated protein kinase 1 (ROCK1) [67]. Another instance is circRNA-CDR1, which promotes adipogenesis and inhibits osteogenic differentiation in osteonecrosis of the femoral head [68]. Furthermore, circRNAs are involved in promoting osteoclastogenesis and bone resorption, underscoring their impact on bone metabolism [74]. CircRNAs modulate this process by influencing key signaling pathways. For example, CircZNF367 enhances osteoclastogenesis by interacting with the FUS RNA-binding protein to stabilize the mRNA coding for cryptochrome circadian regulator 2 (CRY2), thereby increasing osteoclast proliferation and expression of trp RNA-binding attenuation protein (TRAP), nuclear factor of activated T-cells, cytoplasmic 1 (NFATc1), and c-FOS [70]. Additionally, circ_0029463 regulates osteoclast differentiation by promoting RANKL-induced differentiation, by modulating the miR- 134 - 5p/Rab27a axis [10]. Other circRNAs, such as circRNA_009934, circFam190a, and exosomal circ_0000722, also contribute to osteoclast-mediated bone resorption and differentiation [75–77]. Understanding the intricate roles of EVs, circRNAs, and miRNAs in bone biology holds promise for developing novel therapeutic strategies targeting bone diseases and enhancing bone regeneration outcomes.

Interaction between immune cells and bone cells

The interaction between immune cells and bone cells is a crucial aspect of maintaining skeletal health and facilitating the healing process. In particular, immune cells (such as macrophages), play a significant role in this dynamic relationship by secreting various factors that can significantly influence the activity of bone cells. These secreted factors can exert a range of effects, from promoting bone formation to inhibiting bone resorption (depending on the body's specific needs) [78, 79]. For example, macrophages can adopt a pro-healing phenotype, known as the M2 phenotype. In this state, macrophages release anti-inflammatory cytokines and growth factors that create an environment conducive to tissue regeneration and repair [80]. This pro-healing phenotype supports the differentiation and function of osteoblasts, thereby enhancing the bone regeneration process. Additionally, the presence of these healing macrophages can modulate the activity of osteoclasts, the cells involved in bone resorption, ensuring a balanced bone remodeling process [81]. This intricate interplay between immune cells and bone cells highlights the importance of the immune system in bone health, beyond its traditional functions in organism defence and pathogen clearance.

Effects of acute and chronic inflammation on bone regeneration

Inflammation plays a dual role in bone regeneration and its impact varies significantly depending on being acute or chronic. Acute inflammation is a short-term and immediate response to injury or infection, characterized by the rapid influx of immune cells to the affected area [82]. This initial phase of inflammation is crucial for the bone healing process, as it sets the stage for tissue repair and regeneration [83]. During acute inflammation, immune cells such as neutrophils and macrophages release cytokines and growth factors that stimulate the activity of osteoblasts [84]. Additionally, the immune cells help clear debris and pathogens from the injury site, creating a favorable environment for healing. In contrast, chronic inflammation is a prolonged, persistent inflammatory response that can have detrimental effects on bone regeneration [85]. When inflammation becomes chronic, it leads to the continuous recruitment of immune cells and the sustained release of pro-inflammatory cytokines. This persistent inflammatory environment can disrupt the normal bone remodeling process, leading to an imbalance between bone formation and bone resorption. In fact, chronic inflammation is often associated with an increased activity of osteoclasts, the cells that break down bone tissue, which can result in bone loss and weakened bone structure [86]. Conditions such as rheumatoid arthritis and chronic infections are situations in which chronic inflammation adversely affects bone health, impairing the body's ability to effectively regenerate bone tissue [86, 87]. Thus, understanding the distinct effects of acute and chronic inflammation on bone regeneration is essential for developing targeted therapies to enhance bone healing, while mitigating the negative impacts of prolonged inflammation.

Mesenchymal stem cells (MSCs) in bone regeneration

Origin and characteristics of MSCs

MSCs are primarily derived from the bone marrow; however, they can also be found in various other tissues throughout the body, including the adipose tissue (fat tissue) and the periosteum (the outer layer of the bone) [88]. MSCs obtained from the bone marrow are one of the most extensively studied and commonly used stem cell sources in research and clinical applications [89]. They are typically isolated from the bone marrow collected from the hip bone (iliac crest) or other long bones. Bone marrow-derived MSCs have demonstrated functional multipotency, capable of differentiating into osteoblasts, chondrocytes, and adipocytes, under appropriate conditions [89]. MSCs derived from adipose tissue are an excellent choice of cells for bone tissue engineering [90]. They are isolated from liposuction aspirates or surgical waste material from procedures such as abdominoplasty. Adipose tissue-derived MSCs exhibit similar multipotent differentiation capabilities to those of bone marrow-derived MSCs, offering a valuable alternative, due to their convenient accessibility and abundance [90, 91]. Furthermore, periosteum-derived MSCs contribute to bone growth and repair by differentiating into osteoblasts and forming new bone tissue [92, 93]. They play a crucial role in the natural healing response of bones to fractures and other injuries.

Properties of MSCs

MSCs exhibit unique properties that make them pivotal in tissue repair and regeneration, particularly in bone formation. Indeed, MSCs are characterized by their capacity to differentiate into multiple cell types, including osteoblasts (bone-forming cells), chondrocytes (cartilage-forming cells), and adipocytes (fat cells) [94]. Such multipotency is the property that enables MSCs to dynamically respond to local environmental stimuli and to differentiate into osteoblasts [35, 95]. A key feature of MSCs is their ability to self-renew through mitotic divisions, while maintaining their undifferentiated state. This self-renewal capability ensures a sustainable source of progenitor cells that can continuously contribute

to tissue repair and regeneration. In the context of bone regeneration, MSCs proliferate in response to injury signals, thereby substituting damaged tissue and supporting the de novo formation of bone matrix. Beyond their regenerative potential, MSCs exert immunomodulatory effects by interacting with immune cells and regulating inflammatory responses [95, 96]. In particular, MSCs interact with immune cells, regulating immune responses and creating an anti-inflammatory environment [97]. This modulation has the additional benefit of reducing tissue damage and promoting healing in conditions marked by inflammation. Due to their regenerative and immunomodulatory properties, MSCs are of significant interest in clinical applications for bone regeneration. MSCs are also fundamental in the tissue repair process, through the secretion of bioactive molecules, including growth factors, EVs, and cytokines. These paracrine factors stimulate local cells to proliferate, differentiate, and migrate to the injury site, promoting tissue regeneration [98–100]. This trophic support is crucial for the therapeutic efficacy of MSC-based treatments in clinical applications [100–102]. The combination of these properties has led to extensive research into the potential of MSCs for treating conditions such as osteoarthritis, and autoimmune diseases [103, 104]. In particular, within the context of bone repair, MSCs respond to signals in the bone microenvironment by differentiating into osteoblasts, thereby contributing to new bone formation. Their secretion of growth factors and cytokines further supports tissue healing and regulates cellular activities necessary for bone regeneration [101, 105]. MSCs have been explored in therapies for bone defects and non-union fractures, in which techniques including tissue engineering and scaffold-based delivery systems enhance their effectiveness in promoting bone formation and integration [106–110]. Thus, the ability of MSCs to promote tissue repair, modulate immune responses, and facilitate regeneration makes them promising candidates for improving patient outcomes and enhancing their quality of life through regenerative medicine approaches [101, 105]. Moreover, their immunomodulatory function helps to mitigate complications such as chronic inflammation, thereby enhancing the overall success of MSC-based therapies in clinical practice.

Factors influencing MSC differentiation into osteoblasts

MSC differentiation into osteoblasts (osteoblastogenesis) is intricately regulated by a combination of intrinsic genetic factors and extrinsic signals present in the bone microenvironment. These factors collectively orchestrate the commitment of MSCs to the osteogenic lineage and their subsequent maturation into functional bone-forming cells. Therefore, intrinsic factors, such as the genetic profile and cellular signalling pathways, and extrinsic factors, including mechanical and biochemical stimuli from the surrounding microenvironment, are important in promoting osteoblastogenesis.

Intrinsic factors

Key transcription factors play pivotal roles in driving osteogenic differentiation. RUNX2 is known as the master gene regulator of osteoblastogenesis, being essential for initiating this process and regulating the expression of genes involved in bone matrix synthesis, mineralization, and osteoblast maturation [111]. SP7/Osterix acts downstream of RUNX2 and is essential for the progression of osteoblast differentiation, promoting the expression of osteogenic genes including osteocalcin and bone sialoprotein. It is involved in the formation of mineralized bone tissue [112]. While primarily associated with chondrogenesis, the Sox9 transcription factor also influences osteogenic differentiation by regulating the equilibrium between osteoblast and chondrocyte differentiation pathways [113–115]. It interacts with RUNX2 to modulate osteoblast-specific gene expression [116]. These transcription factors work in concert to coordinate the sequential activation of osteogenic genes and the suppression of alternative lineage fates, thereby ensuring the commitment of MSCs to the osteoblastic lineage.

Another crucial factor in regulating osteoblast differentiation is β-catenin, a key mediator of the canonical Wnt signalling pathway. β-Catenin plays an essential role in mesenchymal precursor cells during their transition into Runx2 + Osx + cells and, subsequently, in their differentiation into mature osteoblasts. Deleting the gene encoding β-catenin in Osx + osteoprogenitors prevents their terminal differentiation into mature osteoblasts, instead promoting the expression of markers associated with chondrocytes or adipocytes [117]. Mutations in genes involved in osteogenic differentiation can lead to various diseases, such as those caused by mutations in the RUNX2 transcription factor. In particular, mutations in the *RUNX2* gene can lead to cleidocranial dysplasia (CCD), a rare genetic disorder characterized by defective development of bones and teeth [118]. CCD is typically inherited in an autosomal dominant manner. Individuals with CCD often exhibit a range of symptoms, including underdeveloped or absent clavicles, delayed closure of cranial sutures resulting in a persistently open skull fontanelle, dental abnormalities such as delayed eruption of permanent teeth and supernumerary teeth, and various other skeletal anomalies [119]. The severity of symptoms can vary widely among affected individuals [119].

Extrinsic signals from the bone microenvironment

The bone microenvironment provides crucial extracellular cues that influence MSC behavior and differentiation into osteoblasts through mechanoreceptors and integrin-mediated signalling pathways [120, 121]. Mechanical stimuli, such as compression, tension, and fluid shear stress, induce changes in MSC morphology, cytoskeletal organization, and gene expression profiles that drive osteogenic differentiation.

In addition, the bone microenvironment is enriched in growth factors and cytokines that act as potent inducers of osteogenic differentiation of MSCs [122–124]. BMPs are key regulators of osteogenesis, stimulating MSCs to commit to the osteoblast lineage and promoting bone formation [125–127]. They function by inducing the expression of osteogenic genes (including RUNX2) and enhancing matrix mineralisation. In addition to BMPs, TGF-β-mediated signaling pathways regulate various aspects of osteoblast differentiation, including cell proliferation, ECM production, and osteogenic gene expression [41, 128, 129]. Furthermore, Insulin-like Growth Factor enhances MSC proliferation and differentiation into osteoblasts by activating downstream signalling pathways that regulate cell growth, survival, and metabolism [130]. Importantly, the composition and mechanical properties of the ECM surrounding MSCs provide critical cues for osteogenic differentiation. ECM proteins such as collagen, fibronectin, and glycosaminoglycans interact with integrin receptors on MSCs, initiating intracellular signalling cascades that promote MSC commitment to osteoblast differentiation and consequent bone tissue formation [11, 131–133]. ECM stiffness and topographical features also influence MSC behaviour and lineage commitment [132].

Cellular senescence and the bone microenvironment

The ability of adult somatic stem cells to proliferate and contribute to tissue regeneration and homeostasis can differ between tissues and change with age [134]. As aging progresses, senescence profoundly impacts the bone microenvironment, disrupting its delicate homeostasis and functionality. Senescent cells accumulate with aging and fundamentally alter the cellular landscape within the bone tissue. This accumulation not only impairs normal physiological processes but also significantly contributes to aging-related bone diseases. It has been reported that the replicative ability of MSCs diminishes as the donor ages, with a simultaneous increase in apoptotic cells that show positive staining for senescence-associated β-galactosidase [135], a marker of cellular senescence. Several investigations have shown that MSCs from older donors are more prone to producing nitrous oxide and reactive oxygen species. They also exhibit a decreased capability to neutralize superoxide radicals [136, 137].

Senescent osteoblasts, once primary builders of bone matrix, experience a decline in their bone-forming capacity. This reduction leads to decreased bone density and strength, which are commonly associated with conditions including osteoporosis [138]. Although senescent cells are in a state of growth arrest, they remain metabolically active. Senescence can alter the internal mechanisms of cells and impact the surrounding environment, by secreting a complex array of substances that can influence the behavior and function of non-senescent cells [139]. This increased secretion activity, known as the senescence-associated secretory phenotype (SASP) or the senescence-message secretome (SMS) [140], is a key feature of cellular senescence. Similarly, senescent osteoclasts, responsible for bone resorption and remodeling, may become dysregulated with aging. This dysregulation can lead to abnormal bone resorption processes, disrupting the equilibrium between bone formation and resorption. Consequently, bone remodeling becomes compromised, further contributing to bone fragility and fracture susceptibility. MSCs, also undergo a functional decline as they enter senescence. This decline reduces their ability to differentiate into osteoblasts and supports the regenerative processes essential for maintaining bone integrity [141, 142]. The diminished regenerative capacity of senescent MSCs poses a significant challenge in addressing age-related bone loss and compromised bone healing.

Furthermore, senescent endothelial cells and immune cells within the bone microenvironment contribute to vascular dysfunction and chronic inflammation [143, 144]. These changes exacerbate the progression of age-related bone diseases by impairing nutrient delivery, metabolite detoxification, and immune surveillance within bone tissue.

Unravelling the complex interplay between cellular senescence and the bone microenvironment not only advances our understanding of bone aging but also opens new avenues for developing innovative therapeutic approaches. Thus, strategies aimed at selectively targeting senescent cells, rejuvenating or replacing aged cells with functional counterparts, and enhancing the regenerative potential of MSCs hold promise for restoring bone health and improving the quality of life in ageing populations. These approaches have the potential to promote skeletal health throughout the lifespan, thereby addressing the growing challenge of age-related bone disorders in an ageing global population.

Innovations in bone regenerative therapies

Clinical applications of MSCs

Recent studies have identified specific signaling pathways involved in bone regeneration, highlighting the potential of manipulating the bone microenvironment to enhance MSC functionality. Notably, differences between 2 and 3D culture systems have emerged as key determinants of MSC behavior [145–147]. While traditional 2D cultures provide a controlled environment for studying osteogenesis, they fail to fully replicate the spatial and mechanical cues of the native bone niche. In contrast, 3D culture systems, including organoids and bioreactors, more accurately mimic the in vivo microenvironment, promoting enhanced differentiation and more physiologically relevant cell interactions [148, 149]. In parallel, scaffold-free transplantation methods are emerging as promising alternatives to traditional biomaterial-based approaches [150, 151]. These techniques, which rely on self-assembled MSC aggregates or spheroids, eliminate the need for exogenous scaffolds, while preserving cell–cell interactions and the endogenous production of ECM, potentially improving transplantation outcomes [152, 153].

MSCs have been used in multiple clinical trials to treat bone defects and diseases. Techniques include the direct injection of MSCs and the use of scaffolds seeded with MSCs. In particular, MSC can be applied to treating long bone nonunions [154, 155], which are fractures that fail to heal properly within the expected timeframe, often due to insufficient biological or mechanical conditions [156]. MSCs can be harvested from the patient's bone marrow, expanded in culture, and directly injected into the nonunion site [157, 158]. This approach aims to enhance bone healing by providing a rich source of osteogenic cells that can aid in bridging the fracture gap. Spinal fusion is a surgical procedure used to correct problems with the small bones in the spine (vertebrae) [159, 160]. MSCs have been investigated to improve the success rates of spinal fusions by promoting bone growth and enhancing the integration of the fusion graft [161, 162]. In some studies, MSCs are combined with bone graft materials and implanted at the fusion site to stimulate new bone formation and ensure a more stable and solid spinal structure [163–165]. Osteonecrosis (or avascular necrosis) of the femoral head is a condition where bone tissue in the hip joint dies due to the lack of blood supply, leading to joint pain and collapse. Clinical trials have explored the use of MSCs to treat early-stage osteonecrosis [166–168]. MSCs are injected into the femoral head of subjects affected by osteonecrosis, in order to promote bone regeneration and prevent further tissue degeneration [169, 170]. The goal of this treatment is to restore the blood supply and facilitate the growth of new, healthy bone tissue. MSCs have also shown promise in treating craniofacial bone defects, which can result from trauma, congenital anomalies, or surgical resections. Indeed, MSCs are employed in conjunction with custom-made scaffolds to reconstruct areas of missing bone. These scaffolds provide a supportive structure for the MSCs to adhere and differentiate into bone-forming cells, leading to the regeneration of the craniofacial skeleton [171]. In addition, MSCs have been applied in the dental regeneration field, to repair and regenerate dental tissues, including alveolar bone, periodontal ligament, and dental pulp. Using tissue engineering techniques in dentistry, MSCs derived from teeth can be induced to form a 3D environment for regenerating and restoring a fully functional tooth complex when combined with a biocompatible scaffold and growth factors [172].

Use of biofactors and biomaterials to enhance bone regeneration

Growth factors and biomaterials are crucial in advancing bone regenerative therapies. As we reported above, growth factors, such as BMPs, play a significant role in stimulating bone formation, by promoting the differentiation and activity of osteoblasts. BMPs and other growth factors can be directly delivered to the injury site or incorporated into scaffolds and biomaterials, to provide sustained release and localized effects [173]. Biomaterials, including hydrogels and bioceramics, offer essential structural support and create a conducive environment for cell adhesion, proliferation, and differentiation [174–176]. Hydrogels, known for their high-water content and biocompatibility, can be tailored to mimic the natural ECM, while bioceramics provide the necessary mechanical strength and osteoconductivity to support new bone growth [177–180].

Combined therapies: stem cells and biofactors

Combining MSCs with bioactive factors and scaffolds represents a cutting-edge approach in bone regenerative medicine. This synergistic strategy leverages the regenerative potential of stem cells with the stimulatory effects of growth factors and the supportive role of biomaterials. For instance, scaffolds seeded with MSCs and loaded with BMPs can create an optimal microenvironment that enhances cell viability, proliferation, and differentiation, thereby promoting more efficient and effective bone regeneration [176, 181]. These combined therapies can be tailored to address specific clinical needs, offering personalized treatment options for patients with various bone defects and diseases. By integrating multiple components into a cohesive treatment strategy, researchers and clinicians

can overcome the limitations of single-modality therapies and achieve superior outcomes in bone healing and regeneration.

Most likely, all these aspects aim to improve bone properties by also acting on its microenvironment. A multifunctional therapeutic system for improving the regenerative microenvironment and increasing bone regeneration via photothermal therapy has been recently reported [182]. This system employs smart and multifunctional techniques to create an improved healing environment and speed up bone regeneration, using mild photothermal therapy to enhance these effects. Designing scaffolds that regulate the immune response and promote vascularized bone regeneration is a promising strategy in bone tissue engineering. Recently, Jin et al. reported a study where they fabricated electrospun scaffolds with M2 macrophage-derived exosomes on nanofibrous structures [183]. These scaffolds enhanced cell migration, osteogenic differentiation, and anti-inflammatory macrophage polarization [183]. In mice, they activated fibrosis, angiogenesis, and macrophages; and in rats, they improved vascularized bone formation. Histological analysis showed that these scaffolds regulated angiogenesis, osteoclastogenesis, and osteogenesis, driving implant design for orthopedics and maxillofacial surgery [183]. In addition, a new approach has been introduced for treating challenging wounds, by controlling early inflammation. Notably, promptly shifting the bone microenvironment from a pro-inflammatory to an anti-inflammatory state following an acute immune response could promote bone formation [184–186]. As previously stated, macrophages play a crucial role in responding to inflammation. Therefore, this research utilizes EVs with a high expression level of T-cell immunoglobulin and mucin domain-containing protein 3 (TIM3, known for its immunosuppressive properties) to reshape the initial immune microenvironment of bone injuries, primarily targeting macrophages [187]. These EVs are phagocytosed by macrophages, leading to increased infiltration of TIM3-positive macrophages (TIM3 + macrophages) and M2 subtype polarization. The TIM3 + macrophages exhibit characteristics akin to M2 macrophages and secrete cytokines including IL- 10 and TGF-β1, to modulate inflammation [187]. Thus, engineered EVs overexpressing TIM3 facilitate the release of anti-inflammatory cytokines by inhibiting the p38/mitogen activated protein kinases (MAPK) signaling pathway. They also enhance osseointegration by activating the Bmp2 promoter to stimulate BMP2 secretion from macrophages [187]. Importantly, incorporating these engineered EVs into a hydrogel allows their gradual and continuous delivery, in order to recruit more anti-inflammatory macrophages during the early phases of bone defect repair. This approach effectively regulates the immune microenvironment and mitigates the detrimental effects of excessive inflammation.

Artificial intelligence and bone regeneration

Advances in artificial intelligence (AI), particularly in deep learning, can significantly enhance scientific knowledge and its clinical outcomes in regenerative medicine. As stated, bone tissue engineering seeks to regenerate damaged or lost bone using cells, biomaterials, and growth factors. By analyzing large datasets, predicting molecular interactions, and optimizing treatment strategies, AI can uncover innovative solutions. Since scaffolds provide frameworks for cell attachment, growth, and differentiation, essential in bone tissue engineering, AI could enhance scaffold design by improving their biomechanical properties, porosity, and bioactivity. Techniques including generative adversarial networks (GANs) and reinforcement learning can generate novel scaffold structures based on specific criteria [188, 189]. Additionally, AI-driven models simulate scaffold mechanics under different conditions, optimizing their integrity and function. Integrating experimental data with simulations allows researchers to refine scaffold designs, speeding up the fabrication of customized, patient-specific implants. The choice of biomaterials significantly impacts construct performance and compatibility in bone tissue engineering. Advanced manufacturing techniques for implants also influence the choice of material. Currently, a combination of 3D printing and robotics enables the mass customization of orthopedic implants [190]. Bioprinting has been used to generate patient-specific heart valves [191]. Titanium alloys are prominent materials in bone tissue engineering [192], as well as various polymers of natural or synthetic origin [193]. Polymers are used to fabricate porous frameworks capable of drug release and can enhance the mechanical properties of these materials when used in composites [193]. As stated, electrospinning is another method employed to develop regenerative scaffolds for osteogenesis, mimicking natural bone tissue structures using custom synthetic or biomimetic materials such as metals, ceramics, and polymers [194]. This technique produces scaffolds with large surface areas, optimal fiber spacing for cell exchange and nutrition, and adjustable support. Electrospun fibers can guide cell attachment, regulate differentiation, and promote osteogenesis, by shaping cell morphology [195, 196]. These innovative technologies—electrospinning and 3D bioprinting—enable the creation of multiscale, multicellular tissues and bionic structures with complex cellular arrangements, tissue diversity, and functional

versatility within intricate microenvironments [197]. AI algorithms streamline the screening of biomaterial libraries by predicting properties including degradation kinetics and immunogenicity. Integrated computational models systematically assess interactions among cells, biomaterials, and biological fluids, guiding informed material selection [197]. AI platforms also innovate by discovering new biomaterial formulations tailored for mechanical, chemical, and biological needs. Thus, by analyzing complex datasets, AI accelerates the development of advanced materials including scaffolds, hydrogels, and composites for bone regeneration [94]. The ultimate aim of bone tissue engineering is to translate preclinical research into effective clinical therapies for patients with bone defects or fractures [94]. AI-driven predictive models further enhance personalized treatment plans by considering individual genetic factors, comorbidities, and lifestyle elements. AI can continuously refine treatment protocols, by leveraging clinical data and feedback, fostering adaptive and patient-centered care strategies. In particular, AI could play a fundamental role in assessing the success of bone regeneration by studying the bone microenvironment. AI has been used to analyse and interpret large amounts of medical data It could be used to identify signs indicating whether the bone regeneration process is progressing successfully or not. These algorithms could also develop predictive models based on historical data to forecast the effectiveness of bone regeneration therapies, considering patient characteristics, bone microenvironment conditions, and the materials used. Moreover, AI could optimize therapies by integrating multi-omics data and clinical insights to recommend personalized treatment protocols that maximize the bone regeneration potential, accounting for individual variables including genetics, comorbidities, and lifestyle. Real-time monitoring using AI-powered sensors or implantable devices could provide continuous feedback on the bone regeneration progress, enabling timely adjustments to treatment plans, if necessary. In synthesis, AI holds promise in revolutionizing the monitoring and optimization of bone regeneration, paving the way for more precise, effective, and personalized patient treatments.

Future perspectives and challenges

Future therapies in bone regeneration will most likely increasingly focus on sophisticated methods to modulate the bone microenvironment. This strategy involves the targeted delivery of signaling molecules (such as growth factors, cytokines, and chemokines) directly to the site of injury or bone defect. These therapies aim to enhance the body's natural regenerative processes by precisely regulating the local biochemical environment. Additionally, advanced gene editing techniques, such as CRISPR-Cas9, have been explored to modify the genetic profile of cells within the bone microenvironment. These modifications can upregulate regenerative pathways, improve cell survival, and promote the formation of new bone tissue. Combining these approaches with biomaterial scaffolds and controlled release systems could create a highly favourable environment for bone regeneration, potentially transforming the landscape of orthopedic treatments. In addition, AI can significantly aid and expedite all processes related to bone regeneration.

Despite the promising results observed in preclinical studies, several challenges remain in translating these findings into effective clinical therapies. One of the primary hurdles is ensuring the survival, integration, and functionality of transplanted cells within the host tissue. Many cells do not survive the initial implantation due to immune reactions, lack of nutrients, or unsuitable microenvironmental conditions. Another significant challenge is the regulatory approval process, which can be lengthy and complex, requiring extensive evidence of safety and efficacy. Additionally, the cost of developing and producing these advanced therapies can be prohibitive, limiting their accessibility and widespread adoption. Addressing these challenges requires a multidisciplinary approach, combining advances in biomaterials, cell biology, and regulatory science, to develop cost-effective and clinically viable solutions.

Furthermore, personalized medicine holds the potential to revolutionize bone regeneration therapies by tailoring treatments to the specific genetic, environmental, and lifestyle factors of individual patients. This approach can improve the effectiveness and efficiency of therapies by addressing each patient's unique needs and conditions. For example, genetic profiling can identify specific molecular targets or pathways that are more active in a patient's bone regeneration process, allowing targeted therapies to be developed. Additionally, personalized 3D-printed scaffolds can be designed to match the exact shape and size of a patient's bone defect, improving the integration and functionality of the regenerative graft. By leveraging advances in genomics, biomaterials, and computational modeling, personalized medicine can make bone regeneration therapies more effective, reducing recovery times and improving patient outcomes. Therefore, to facilitate the successful implementation of these advanced strategies, a structured research and clinical roadmap is essential. There is the need of a: 1) preclinical optimization, by refining biomaterial composition, MSC expansion techniques, and delivery methods in animal models. There is also the need of a 2) standardization

and regulatory approval, through the development of good manufacturing practice (GMP)-compliant cell culture and scaffold production protocols. 3) Scalable and cost-effective production is also needed, through the integration of automated bioprocessing and AI-driven design, to reduce costs and improve reproducibility. 4) Personalized treatment strategies also need to be developed, through the Implementation of AI-based predictive models for patient stratification, enabling personalized biomaterial selection and regenerative protocols. 5) Clinical trials and real-world applications, such as multi-center trials assessing safety, efficacy, and long-term outcomes in diverse patient populations are also required.

Conclusion

This review highlights the significant roles of MSCs and cellular communication in bone regeneration. MSCs are crucial in this process, due to their ability to differentiate into osteoblasts and other essential cell types. The present review underscores the importance of cellular communication, where factors secreted by immune cells and other cell types modulate MSC activity, influencing bone formation and resorption. Recent discoveries have shed light on these interactions, opening new therapeutic avenues by manipulating the bone microenvironment and enhancing MSC functionality. Understanding the bone microenvironment, including the regulatory roles of factors secreted by immune cells and other entities, will yield new therapeutic targets, improving bone healing and regeneration interventions. The integration of advanced biomaterials, gene editing technology, and controlled delivery systems promises more targeted and efficient bone regeneration therapies. Therefore, the future of bone regeneration will depend on combining multidisciplinary approaches from stem cell biology, biomaterials science, genetics, and clinical medicine. Innovations in biotechnology and personalized medicine are needed for overcoming current limitations. Personalized treatments offer more effective solutions, using patient-specific stem cells and custom-designed scaffolds. Advances in gene editing and molecular biology could further enhance the regenerative capacity of MSCs. In summary, the future of bone regeneration lies in multidisciplinary collaboration and technological innovation.

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Author contributions
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Declarations

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