



Review

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Role of the immune microenvironment in bone regeneration and progress in immune-modulating bone nanobiomaterials

Rong'an LI*, Jiakang YANG*, Wenjun DUAN, Kexin YANG, Katong LO, Qianming CHEN[✉], Baixiang WANG[✉]

Stomatology Hospital, School of Stomatology, Zhejiang Provincial Clinical Research Center for Oral Diseases, Key Laboratory of Oral Biomedical Research of Zhejiang Province, Engineering Research Center of Oral Biomaterials and Devices of Zhejiang Province, Zhejiang University School of Medicine, Cancer Center of Zhejiang University, Hangzhou 310000, China

Abstract: Inflammation is a key physiological process in the regeneration of bone tissue following injury. The acute inflammatory response, along with the timely resolution of inflammation, is essential for effective bone tissue repair. Exacerbation of either acute or chronic inflammation can lead to impaired bone regeneration, which is closely associated with interactions between immune cells and bone-related cells, as well as the regulatory roles of various inflammatory cytokines. In this review, we discuss the role of the immune microenvironment in bone regeneration and the negative impact of dysregulated inflammation on bone regeneration, and highlight on the need for timely elimination of inflammation. Additionally, the application of nanobiomaterials with immunomodulatory function in the treatment of inflammatory bone defects is discussed to clarify its current challenges and the future direction of its development.

Key words: Immune microenvironment; Bone defect; Bone regeneration; Immunomodulation; Bone nanobiomaterial

1 Introduction

Bone defects can arise from various causes such as trauma, infection, tumors, degenerative conditions, and congenital diseases. Unlike many other tissues, bone has a robust intrinsic healing capacity, allowing it to regenerate without the formation of fibrous scars. The process of fracture healing or bone defect reconstruction recapitulates skeletal development and can be regarded as a form of tissue regeneration, namely bone regeneration (Marsell and Einhorn, 2011). Nevertheless, bone regeneration occasionally encounters obstacles attributed to factors including infection, abnormal movement, and compromised blood supply (Claes et al., 2012). Approximately 5%–10% of injuries

result in compromised bone healing, characterized by delayed healing, inadequate regeneration, or even complete failure to heal (Marsell and Einhorn, 2011; Einhorn and Gerstenfeld, 2015).

On the basis of biological characteristics, the bone healing process can be categorized into two main types: primary (direct) healing and secondary (indirect) healing (Marsell and Einhorn, 2011). Direct healing is relatively infrequent and necessitates the absence of any gap between the ends of the bone defect and a stable fixation. In the case of fractures in flat bones, such as the skull, direct healing occurs wherein progenitor cells directly undergo osteoblastic differentiation, leading to intramembranous ossification and subsequent formation of a new bone matrix (Frost, 1989). Indirect healing, the more prevailing form of bone regeneration, is observed in most cases and involves both intramembranous and endochondral osteogenesis (Saul and Khosla, 2022). Regardless of the injury location and healing type, bone tissue regeneration adheres to a defined process characterized by three sequential and interconnected phases: inflammation, regeneration, and remodeling. In a continuous and overlapping manner, bone tissue undergoes a series of

✉ Baixiang WANG, wangbaixiang@zju.edu.cn

Qianming CHEN, qmchen@zju.edu.cn

* The two authors contributed equally to this work

✉ Baixiang WANG, <https://orcid.org/0000-0002-7034-4189>

Qianming CHEN, <https://orcid.org/0000-0002-5371-4432>

Rong'an LI, <https://orcid.org/0009-0007-5162-6360>

Jiakang YANG, <https://orcid.org/0000-0003-2922-7615>

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changes, including endochondral ossification, intramembranous ossification, and formation of adherent bone, which are tightly controlled by systemic and local secretory factors (Marsell and Einhorn, 2011; Claes et al., 2012). The entire process of bone tissue healing and the cells and cytokines involved in each process are shown in (Fig. 1). The severity of trauma, stability of the mechanical microenvironment, interplay among osteoblasts, inflammatory cells, and inflammatory factors, and post-traumatic blood flow reconstruction can significantly affect the outcome of bone healing (Loi et al., 2016; Bahney et al., 2019). However, the precise mechanisms underlying the interactions among various cells and molecules involved in bone regeneration under both physiological and pathological conditions remain incompletely understood.

The immune system, which comprises immune cells and associated cytokines, actively contributes to the regulation of bone homeostasis and plays a crucial role in tissue repair and regeneration (Weitzmann, 2017). During the initial phases of a bone defect, an acute inflammatory response is imperative for bone healing. This inflammatory response typically persists for several days and then gradually diminishes. The

cells involved in this response, along with released cytokines, collaborate to establish a pro-regenerative immune microenvironment. Dysregulation of inflammation, including excessive or insufficient secretion of inflammatory factors and prolonged duration, can result in an abnormal immune microenvironment and impaired bone regeneration (Claes et al., 2012; Bahney et al., 2019; Saul and Khosla, 2022). Recent research suggests that timely resolution of inflammation following the pro-inflammatory phase may be an effective therapeutic strategy to improve bone tissue healing (Kwon et al., 2017; Mahon et al., 2020). The implementing of active anti-inflammatory strategies serves dual purposes: preventing a persistent chronic inflammatory state and creating a pro-regenerative microenvironment. Consequently, this requires that bone biomaterials not only function as scaffolds for facilitating new bone formation, but also act to directly or indirectly regulate the inflammation process.

In this review, we aim to elucidate the significance of the immune microenvironment in bone regeneration by highlighting the detrimental effects of an aberrant immune process, particularly long-term chronic inflammation, on new bone formation, and the need for timely resolution of inflammation. The application

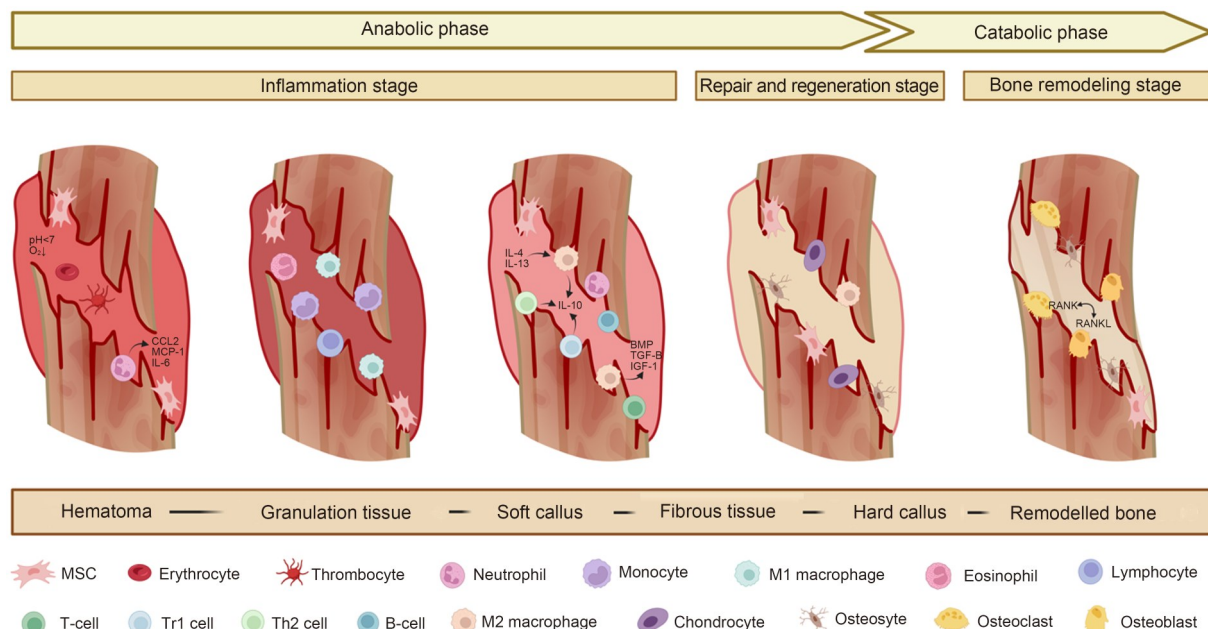


Fig. 1 Stages of bone healing. The two main metabolic stages of bone tissue healing (synthetic metabolism and catabolism) overlap with three biological stages (inflammation stage, repair and regeneration stage, and bone remodeling stage). The sequence progresses from hematoma to granulation tissue, then to cartilage and hard callus formation, culminating in new bone generation. Immune cells and cytokines are key regulators in this regenerative process. MSC: mesenchymal stem cell; Tr1: T regulatory type 1; Th2: T helper cell type 2. Created with BioRender.com.

and development of bone nanobiomaterials with immune regulatory capabilities for the treatment of inflammatory bone defects are also discussed.

2 Role of the immune microenvironment in bone regeneration

2.1 Acute inflammation after bone damage

Acute inflammation is recognized as the primary stage of bone tissue healing, and is characterized by the infiltration of immune cells, clearance of cellular debris, and secretion of cytokines and growth factors (Julier et al., 2017). Following a bone defect or injury, the rupture of surrounding blood vessels leads to the formation of a fibrin-rich hematoma, creating a hypoxic and acidic microenvironment. This hematoma contains inflammatory cells and cytokines derived from peripheral blood, serving as the initial framework for bone regeneration (Kolar et al., 2010, 2011; Yuasa et al., 2015). The first cells recruited are neutrophils, which are attracted to the site by dead cells and debris. Within the first few hours post-injury, neutrophils rapidly accumulate and act to eliminate invading pathogens. Additionally, they secrete various chemokines, including C-C motif chemokine ligand 2 (CCL2), also known as monocyte chemoattractant protein-1 (MCP-1), and interleukin-6 (IL-6), to recruit other inflammatory cells and initiate immune responses (Chung et al., 2006; Yang and Liu, 2021). Research reports indicate that reducing the number of neutrophils during anti-inflammatory treatment in the early stages of bone healing can result in severe impairment of bone regeneration, including a significantly decreased new bone mass and impaired mechanical properties of the fracture callus (Kovtun et al., 2016). Therefore, neutrophils are indispensable in the early stages of bone repair.

Approximately 24–48 h after bone injury, neutrophils are replaced by monocytes, which differentiate into classically activated macrophages (CAMs), also known as pro-inflammatory M1 macrophages, under the stimulation of interferon- γ (IFN- γ) and other pro-inflammatory chemokines (Ellis and Beaman, 2004; Saul and Khosla, 2022). The M1 macrophages express and release high levels of pro-inflammatory cytokines, mainly including IL-1, IL-6, and tumor necrosis factor- α (TNF- α). They also secrete chemokines

such as C-X-C motif chemokine ligand 1 (CXCL1), CXCL2, and CCL5, to further recruit lymphocytes and granulocytes (Arango Duque and Descoteaux, 2014; Huang et al., 2017). Effector T cells, including T helper cell type 1 (Th1) and Th17, rapidly increase in number and secrete pro-inflammatory cytokines. IL-17 produced by Th17 induces the expression of a receptor activator of nuclear factor- κ B (NF- κ B) (RANK) ligand (RANKL) on osteoclast precursors, bone marrow stromal cells, and osteoblasts, thereby promoting osteoclast formation and inhibiting osteoblast differentiation (Yang and Liu, 2021). These pro-inflammatory cytokines reach their peak within a short period, initiating the early innate immune response while simultaneously promoting the migration and proliferation of mesenchymal stem cells (MSCs) and neovascularization.

In the process of bone regeneration, cytokines often form a complex regulatory network through synergistic interactions. Under normal conditions, a transient increase in TNF- α concentration during the early stages of bone healing has a positive effect on bone regeneration. It mainly promotes the migration and recruitment of MSCs by upregulating intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) (Fu et al., 2009). However, an increase of TNF- α concentration at inappropriate time points is a manifestation of a pathological state. IL-1 is a key factor in osteoclast differentiation and formation, facilitating the early clearance of damaged tissue. Additionally, it regulates the expression of cyclooxygenase (COX), which can promote angiogenesis and the differentiation of MSCs into osteoblasts (Cottrell and O'Connor, 2010). IL-1 and TNF- α work synergistically to accelerate the formation of cartilaginous callus, thereby stabilizing the fracture site. IL-6 enhances the expression of osteogenic proteins Runt-related transcription factor 2 (RUNX2) and osteocalcin (OCN), promoting osteoblast differentiation, while also stimulating the release of vascular endothelial growth factor (VEGF) (Yang and Liu, 2021). Compared to wild-type mice, *IL-6* knockout mice exhibit decreased bone density, lower bone tissue mineralization, and a significantly delayed maturation of the callus (Yang et al., 2007). Therefore, artificial depletion of M1 macrophages with reduced secretion of IL-6 and TNF- α significantly impairs femur fracture healing in mice (Hozain and Cottrell, 2020). In general, initial acute

inflammation stimulates angiogenesis and promotes MSC proliferation and differentiation, which is crucial for subsequent bone tissue healing. Therefore, early and excessive anti-inflammatory treatment can be detrimental to new bone formation.

2.2 Inflammation resolution in bone regeneration

The process of inflammation resolution is coordinated by a variety of immune cells and different anti-inflammatory mediators and cytokines. The proinflammatory factors mentioned above can initiate the resolution of inflammation and create a pro-regenerative environment. IL-6, for example, can promote the polarization of M1 macrophages into alternatively activated macrophages (AAMs), namely anti-inflammatory M2 macrophages, and can induce cluster of differentiation 4-positive (CD4⁺) T cells to produce IL-4, polarizing them into Th2, or differentiate into T regulatory type 1 (Tr1) cells to secrete IL-10 (Jin et al., 2013; Fernando et al., 2014). Research has indicated that M2 macrophages, regulatory T cells (Tregs), and Th2 are involved mainly in the phase of inflammation resolution (Schlundt et al., 2018, 2019). M1 and M2 macrophages exhibit distinct cytokine profiles, representing different immune environments. Their dynamic interconversion ensures a balanced regulatory mechanism, playing a dual role in bone tissue regeneration (Chen et al., 2017a). The presence of M1 macrophages in the early stage aids in pathogen clearance, the activation of antimicrobial activity, and the induction of initial angiogenesis. However, prolonged M1 macrophage dominance can lead to chronic inflammation, ultimately inhibiting osteogenesis. Timely polarization toward the M2 phenotype supports tissue repair, whereas premature M2 macrophage polarization may reduce bone mineralization levels. Notably, the M1/M2 macrophage-dominated phases of tissue healing are not entirely independent. Therefore, under pathological conditions, precise regulation of the M1/M2 macrophage polarization time window is crucial for promoting bone regeneration.

As the main group of macrophages involved in the late stage of bone regeneration, M2 macrophages are involved mainly in bone repair and tissue regeneration, including the release of anti-inflammatory cytokines, participation in angiogenesis, and promotion of extracellular matrix (ECM) synthesis and cell proliferation (Pajarinen et al., 2019). IL-10, as one of the

most important pleiotropic immunomodulatory cytokines, plays a critical role in suppressing innate and adaptive immune responses triggered by pathogens or self-antigens. Research has shown that IL-10 promotes the maintenance of bone mass by inhibiting osteoclast bone resorption and regulating osteoblast bone formation, while reducing IL-10 levels in mice leads to significantly accelerated alveolar bone resorption and reduced bone formation (Zhang et al., 2014). In addition to IL-10, the anti-inflammatory factors IL-4 and IL-13 released by CD4⁺ T cells, granulocytes, and natural killer (NK) cells cooperate with IL-10 to stabilize the polarization of macrophages into the M2 type, and promote the osteogenic differentiation and mineralization of bone marrow stromal cells (BMSCs) (Mahon et al., 2020).

M2 macrophages also secrete growth factors and chemokines, including bone morphogenetic protein (BMP), transforming growth factor- β (TGF- β), and insulin-like growth factor (IGF) (Pajarinen et al., 2019). They can inhibit osteoclast formation, promote collagen deposition, and promote the differentiation and proliferation of osteoblasts, in addition to regulating cartilage differentiation (Schlundt et al., 2018). Growth factors such as TGF- β and IGF-1 can directly regulate immune cells and act on endothelial cells or osteoblasts to promote angiogenesis and bone formation (Newman et al., 2021). Inflammatory cytokines such as IL-6 have been shown to interact with TGF- β and enhance TGF- β signaling. TGF- β , in turn, promotes the differentiation of T cells into Tregs and the polarization of macrophages into the M2 phenotype, while also inhibiting mast cell proliferation, reducing the expression of TNF- α , and promoting inflammatory regression (Shull et al., 1992; Oh and Li, 2013). Tregs play crucial positive regulatory roles in bone healing and regeneration. By secreting IL-10 and TGF- β , they suppress Th1 and Th17 responses and inhibit osteoclast differentiation. In addition, they enhance the osteogenic potential of MSCs and osteoblast precursors (Lei et al., 2015). Zaiss et al. (2010) found that transgenic mice with elevated Treg levels exhibited higher bone density and lower bone resorption than wild-type controls (Zaiss et al., 2010).

The next stage involves bone tissue healing, repair, and regeneration, during which cartilage forms and gradually increases, followed by mineralization and absorption (Gerstenfeld et al., 2003; Saul and

Khosla, 2022). The synthetic metabolism phase of bone healing involves the apoptosis of chondrocytes. This is followed by the stage in which catabolic metabolism activity dominates. The callus tissue gradually diminishes, eventually transitioning into the bone remodeling stage, which can persist for several months or even years (Einhorn and Gerstenfeld, 2015). This is achieved mainly through a delicate balance between the absorption of hard callus tissue by osteoclasts and the deposition of layered bone by osteoblasts (Marsell and Einhorn, 2011; Saul and Khosla, 2022; Duda et al., 2023).

Overall, the crosstalk between the immune system and bone plays a critical role in bone regeneration, as illustrated in Fig. 2.

3 Effect of an abnormal immune microenvironment on bone regeneration

The level and duration of the inflammatory response are critical to bone regeneration. Excessive or prolonged inflammation increases the risk of impaired

bone healing (Claes et al., 2012; Maruyama et al., 2020; Newman et al., 2021). Both acute and chronic inflammation share several common characteristics, including substantial upregulation of pro-inflammatory factors such as TNF- α and IL-6 (Claes et al., 2012; Hurtgen et al., 2016; Lim et al., 2017). Intervention measures targeting TNF- α have been shown to mitigate the detrimental effects of excessive inflammation on bone regeneration. Clarifying the specific role of a dysregulated immune microenvironment on bone regeneration and intervening by targeting relevant immune cells and cytokines could be effective therapeutic strategies for treating inflammatory bone defects.

3.1 Effect of excessive acute inflammation on bone regeneration

Studies have shown that excessive acute inflammation, which is frequently associated with polytrauma, can result in the overproduction and rapid release of pro-inflammatory cytokines or chemokines (Relja et al., 2020). It first reduces neutrophil apoptosis, leading to neutrophil overaccumulation at the bone defect site, which in turn impairs bone healing (Bastian et al.,

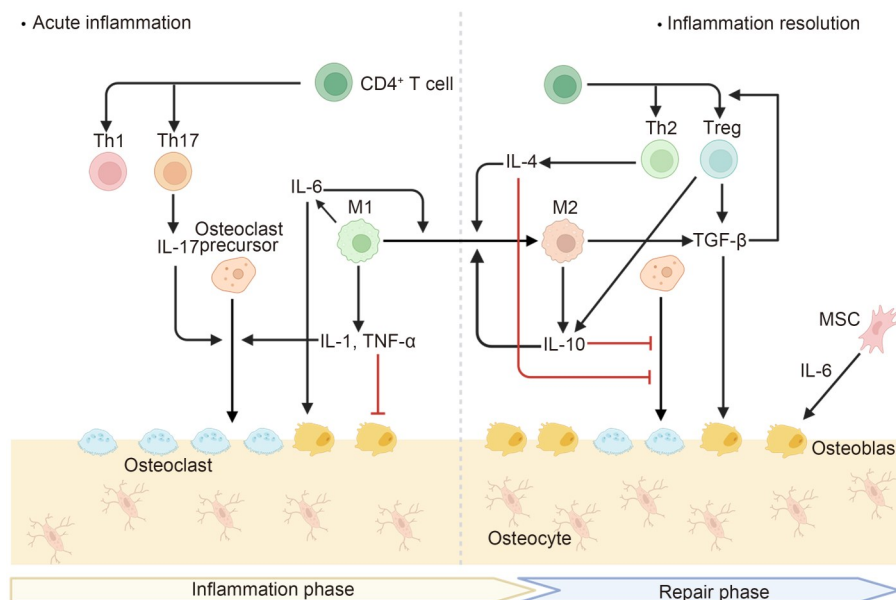


Fig. 2 Interactions between the immune and skeletal systems in bone regeneration. The diagram shows how immune cells regulate the balance between inflammation and bone metabolism in a temporal manner. In the early stage of inflammation, T helper cell type 1 (Th1), Th17, and M1 macrophages are activated and release pro-inflammatory factors such as interleukin-1 (IL-1) and tumor necrosis factor- α (TNF- α), which aggravate the inflammatory response and promote the differentiation of osteoclast precursor cells into osteoclasts and enhance bone resorption. In the stage of inflammation resolution, Th2, regulatory T cells (Tregs), and M2 macrophages are the main cells that jointly release anti-inflammatory factors such as IL-10, slow down the inflammatory response, initiate osteogenic differentiation, and promote tissue repair. MSC: mesenchymal stem cell; TGF- β : transforming growth factor- β . Created with BioRender.com.

2011). In mice with craniocerebral injury combined with femur fracture, the levels of IL-6 and MCP-1 increased abnormally, in addition to a decrease of neutrophil apoptosis (Weckbach et al., 2013). Moderate amounts of IL-6 can shorten the maturation time of fracture calluses and promote the formation of new bone and adipose tissue within bone defects, whereas excessive IL-6 exerts negative effects on bone regeneration (Huang et al., 2018; Takeuchi et al., 2021). Specifically, IL-6 can stimulate cells like Th17 to induce the formation of RANKL, enhance the differentiation of bone marrow-derived macrophages (BMMs) into osteoclasts, and suppress the messenger RNA (mRNA) expression of *RUNX2*, osterix (*OSX*), and *OCN* to inhibit osteoblast differentiation (Hoff et al., 2017; Takeuchi et al., 2021). The CD4⁺ T cells promote the osteogenic differentiation of human BMSCs, whereas in severely traumatized patients, the CD4⁺/CD8⁺ T cell ratio is reduced. These findings suggest that the systemic inflammatory state of patients with polytrauma could directly influence the immune microenvironment within the bone defect area, leading to a longer healing time.

3.2 Effect of persistent chronic inflammation on bone regeneration

A number of studies have shown that persistent chronic inflammation is detrimental to bone tissue regeneration. The continuous production of pro-inflammatory cytokines within the immune microenvironment increases osteoclast activity, inhibits bone formation by osteoblasts, and affects the final bone remodeling stage (Bastian et al., 2011; Claes et al., 2012; Loi et al., 2016; Pajarinen et al., 2019). During chronic inflammation, persistent activation of M1 macrophages sustains elevated levels of pro-inflammatory cytokines including IL-1, IL-6, and TNF- α , which can stimulate osteoblasts or activated T cells to release RANKL. RANKL can interact with RANK on the surface of osteoclasts to activate the NF- κ B pathway and induce osteoclast differentiation and activation (Hardy and Cooper, 2009; Claes et al., 2012; Lim et al., 2017). The NF- κ B pathway, persistently activated by inflammatory cytokines, also regulates their expression and is a common therapeutic target in chronic inflammatory diseases such as rheumatoid arthritis (RA) (Jimi et al., 2019). Elevated levels of pro-inflammatory cytokines directly impair bone regeneration. For

instance, IL-1 enhances bone resorption by promoting osteoclast adhesion to the surface of calcified bone trabeculae, while reducing the proliferation, migration, and differentiation of BMSCs (Martino et al., 2016; Yang and Liu, 2021).

Many local or systemic diseases closely associated with chronic inflammation, such as periodontitis, diabetes, RA, and physiologic aging, are prone to poor healing of bone defects (Timmen et al., 2014; Zhang et al., 2014; Saul and Khosla, 2022; Tanios et al., 2022; Kushioka et al., 2023). Periodontitis is widely recognized as a type of inflammatory disease associated with microecological imbalance (Jacobs et al., 2024; Fang et al., 2025). It is related to the plaque biofilms and the dysbiosis that occurs in periodontal tissues (Sadek et al., 2023). The sustained immune response of the host to pathogens leads to abnormal recruitment of inflammatory cells and chemokines. Chronic inflammation in periodontitis cannot resolve normally and the activity of osteoclasts in the alveolar bone is significantly increased, ultimately resulting in irreversible bone resorption. Research has found that TNF- α , IL-1, and IL-6 are the main pro-inflammatory cytokines in the periodontitis environment that disrupt alveolar bone remodeling and cause bone resorption (Zheng et al., 2017). For example, aberrantly elevated IL-1 levels suppress BMP-mediated osteogenic differentiation by activating the NF- κ B and mitogen-activated protein kinase (MAPK) signaling pathways (Sadek et al., 2023). In the presence of IL-1, TNF- α can also stimulate osteoclast differentiation through mechanisms independent of the RANKL–RANK interaction (Kobayashi et al., 2000). Activated neutrophils can upregulate the expression of RANKL, while T cells and B cells activated by neutrophils are also the main sources of RANKL in periodontitis (Mantovani et al., 2011; Sadek et al., 2023). Both TNF- α and IL-1 down-regulate the levels of osteoprotegerin (OPG), which can suppress osteoclast genesis indirectly by competitively binding to RANK and inhibiting the effect of RANKL. Consequently, the RANKL/OPG ratio is abnormally elevated in periodontitis, which promotes the proliferation, maturation, and differentiation of osteoclasts, disrupting the equilibrium between bone resorption and bone formation, and ultimately impairing normal bone remodeling (Liu et al., 2003; Huang et al., 2020). The osteosclerotic protein, encoded by the sclerostin (*SOST*) gene, is a glycoprotein that is secreted by bone cells. In patients with chronic

periodontitis, the content of this protein in the gingival crevicular fluid is significantly greater than that in healthy individuals (Rezaei Esfahrood et al., 2018; Huang et al., 2020). It can negatively mediate osteoblast genesis by interrupting Wnt/ β -catenin signaling, whereas in *SOST* knockout mice, increased bone thickness, bone mineral density, and bone mechanical strength are observed (Kuchler et al., 2014; Huang et al., 2020). The pathological factors and inflammatory stimuli present in the environment of periodontitis can induce senescence and dysfunction of osteoblasts. This phenomenon, known as inflammatory aging, has detrimental effects on bone remodeling and disrupts the normal balance of bone homeostasis (Franceschi et al., 2000). Further details on this topic are discussed below.

Patients with diabetes also exhibit weak bone healing and delayed fracture repair, with a greater risk of complications such as nonunion (Jiao et al., 2015; Ferrari et al., 2018; Tanios et al., 2022). High glucose can enhance the expression of pro-inflammatory factors, promote early cartilage absorption, and reduce the number of BMSCs (Ding et al., 2020). It is widely recognized that this process is mediated by the up-regulation of the pro-inflammatory cytokine TNF- α , which has been associated with a significant increase in both the number and activity of osteoclasts, particularly in diabetic patients (Alblowi et al., 2009; Saul and Khosla, 2022; Tanios et al., 2022). Elevated levels of TNF- α can stimulate chondrocyte apoptosis, leading to premature resorption of cartilage healing tissue and decreased biomechanical stability of the newly formed bone tissue (Alblowi et al., 2009; Pacios et al., 2013; Newman et al., 2021). Indeed, studies have indicated that elevated levels of TNF- α mediate BMSC damage in a high-glucose microenvironment via the forkhead box protein O1 (FOXO1) pathway, inhibiting their proliferation and impairing osteogenic differentiation (Ko et al., 2015; Fijany et al., 2019; Zhang et al., 2022). In contrast, knockdown of FOXO1 can improve this situation. Furthermore, diabetes significantly reduces angiogenesis within the region of new bone formation, affecting the reconstruction of blood flow in newly formed bone. Although, TNF- α was previously reported to be a pro-angiogenic cytokine, dysregulated TNF- α in a diabetic setting increases apoptosis of endothelial cells by inducing and activating caspase-3 (Sasi et al., 2012; Lim et al., 2017).

Meanwhile, a decrease in the expression of VEGF is observed in the bone regeneration area, and the dys-regulated TNF- α also reduces the ability of endothelial cells in response to growth factors (Lim et al., 2017). Diabetes, mainly type 2, has been identified as an inflammatory disease (Donath and Shoelson, 2011). In addition to TNF- α , the levels of other pro-inflammatory factors, including IL-1 β , IL-6, and IL-18, are also significantly elevated, further activating the NF- κ B pathway, amplifying inflammation, and delaying its resolution (Jiao et al., 2015; Ko et al., 2019). Research has shown that excessive NF- κ B pathway activation decreases TGF- β 1 expression in endogenous skeletal stem cells (SSCs), causing defective polarization and dysfunction of macrophages. However, blocking the NF- κ B pathway or intervention with exogenous TGF- β 1 can rescue bone regeneration within a high-glucose microenvironment (Ko et al., 2019).

Physiological aging can be considered a chronic inflammatory state. A defining characteristic of senescence is the persistence of a low-grade chronic inflammatory state, which can be termed “inflammaging,” which is characterized by elevated levels of multiple pro-inflammatory cytokines in tissues and circulation (Franceschi et al., 2000; Goodnough and Goodman, 2022; Kushioka et al., 2023; Sadek et al., 2023). A related study found that the inflammation level in a bone defect area was obviously downregulated two weeks after fracture in young mice, whereas in older mice it remained at a high level. This effect may be correlated with enhancement of the myeloid differentiation primary response protein 88 (MyD88)-mediated Toll-like receptor (TLR) signaling pathway (Lopez et al., 2022). Cellular senescence is a hallmark of aging, and senescent cells can inhibit the proliferation and function of peripheral cells by secreting the senescence-associated secretory phenotype (SASP) (Josephson et al., 2019). The SASP contains a range of pro-inflammatory mediators, such as TNF- α , IL-1, and IL-6 (Balistreri et al., 2013; Kushioka et al., 2023). These pro-inflammatory cytokines initiate dysfunction in SSCs by activating the NF- κ B signaling pathway (Salminen et al., 2012; Goodnough and Goodman, 2022). As a key driver of the SASP, NF- κ B also enhances SASP secretion. This vicious circle results in senescence among the adjacent cells and generates a continuous inflammatory microenvironment that does not subside easily (Olivieri et al., 2018). Research has shown that

modifications in the inflammatory microenvironment, rather than age alone, significantly rescue the decrease in the number and functionality of SSCs (Josephson et al., 2019). Therefore, the use of mild anti-inflammatory drugs to intervene in the pro-inflammatory state and block the vicious circle may restore the regenerative potential of senescent SSCs and improve the healing of bone defects. Besides, the aging process can affect the polarization balance between M1 and M2 macrophages, impeding the timely transition of macrophages from a pro-inflammatory to an anti-inflammatory state (Pajarinen et al., 2019; Kushioka et al., 2023). In elderly Sprague-Dawley (SD) rats, the number of M2 macrophages in the fracture hematoma decreased significantly and the regeneration of blood vessels in the fracture scab weakened. Transplanting CD14⁺ macrophage precursor cells in elderly rats partially improved bone regeneration at six weeks post-fracture, promoting tissue vascularization and reducing fibrosis (Löffler et al., 2019).

In summary, although periodontitis, diabetes, and aging arise from distinct etiologies, they share remarkably similar pathological features that contribute to impaired bone healing. Persistent microbial stimulation in periodontitis, accumulation of advanced glycation end-products (AGEs) under hyperglycemic conditions, and chronic low-grade inflammation associated with aging collectively maintain a sustained activation of the immune microenvironment. These conditions lead to immune dysregulation and increased oxidative stress, such as polarization of macrophages toward the pro-inflammatory M1 macrophages phenotype, increased T cell activation, and excessive accumulation of reactive oxygen species (ROS), all of which exacerbate local tissue damage and inhibit osteogenesis. Moreover, aberrant activation of signaling pathways such as NF- κ B pathway promotes the release of pro-inflammatory cytokines, osteoclast activation, and vascular injury. Therefore, these pathological states impair bone repair and regeneration through common mechanisms involving chronic inflammation, immune imbalance, oxidative stress, and stem cell exhaustion.

4 Immunomodulatory bone nanobiomaterials

4.1 Characteristics and advantages of nanobiomaterials

Biomaterials have been widely used in bone tissue engineering. An ideal bone biomaterial should satisfy

the following requirements: osteoinductive capability, biocompatibility, angiogenic promotion, mechanical properties comparable to natural bone, and controlled biodegradation (Yu et al., 2025). Functionally, they can not only provide spatial support for regeneration, but also serve as carriers for the delivery of drugs, growth factors, genes, and cells (Lee et al., 2019).

Bone biomaterials can be divided into nano-, micro- and macro-materials according to their structural morphology (Newman et al., 2021). Bone nanobiomaterials, as a rapidly developing field in recent years, have shown favorable prospects for clinical application, especially for treating irregular bone defects. The presence of a porous microstructure is essential for biomaterials to promote good osteoinductivity and osteoconductivity (Wei et al., 2025). Owing to their nanoscale size, drug carriers can be efficiently internalized by cells through endocytosis (Gulati et al., 2022). Compared to micrometer-size materials, nanomaterials have increased specific surface areas, better biodegradability and biological activity, and more bioactive sites (Bramhill et al., 2017). For example, 10% mass fraction nanoscale bioactive glass particles can provide significantly more sites for the nucleation of hydroxyapatite than their microscale counterparts (Fratzl et al., 2004). The nanoscale can also significantly improve the mechanical properties of biomaterials, and increase their hydrophilicity and protein adsorption ability, while their nanomorphology can influence the proliferation and function of adherent cells (Cao et al., 2014; Davison et al., 2015; Shemesh et al., 2017).

Increasing evidence suggests that implantable nanomaterials can cause additional inflammatory reactions at the site of injury, namely a foreign body response (FBR) (Newman et al., 2021). The production of macrophages and foreign body giant cells (FBGCs) can induce osteoclast genesis, leading to bone loss (Trindade et al., 2016). Thus, biomaterials with the ability to promote osteoblast differentiation or vascularization alone have been gradually failed to meet the needs of complex clinical bone defect cases, especially in patients with associated diseases including diabetes, RA, and periodontitis. The design of a biomaterial should consider its interactions with the immune system to minimize adverse inflammatory reactions and the ability to regulate the immune microenvironment of bone regeneration via the material itself or the specific drug molecules it is carrying (Newman et al., 2021; Lopez et al., 2022). Qiao et al. (2021) reported

that in a rat femoral defect model, the implantation of alginate saline gel containing Mg^{2+} induced the production of multinucleated giant cells (MNGCs), which highly exhibited CD206 and showed positive tartrate-resistant acid phosphatase (TRAP) staining. These MNGCs differ from M2 macrophages and osteoclasts, but their apparent pathological involvement in the microenvironment surrounding bone biomaterials could involve M2 macrophage-like wound healing and inflammation-terminating molecules, such as chitinase 3-like protein 1 (Ym1), arginase-1 (Arg1), and arachidonic acid 15-lipoxygenase (Alox15), which could reduce immune dysfunction caused by FBR (Katsuyama et al., 2015; Qiao et al., 2021). Studies have shown that the loss of macrophages induced by clodronate-encapsulated liposomes can effectively reduce the degree of FBR mainly by blocking monocyte infiltration, neovascularization, and material fibrosis (Mooney et al., 2010). The physical properties of the implant, such as shape, size, and texture, can also be modified to regulate immune cells and reduce the redundant inflammatory response (Anderson et al., 2008; Veis and Vegas, 2019).

4.2 Pathways of action of immunomodulatory bone nanobiomaterials

Currently, most bone nanobiomaterials function by directly targeting osteoblasts or osteoclasts. For instance, Wei et al. (2025) established a nanocomposite hydrogel system consisting of alginate methacrylate and curcumin-encapsulated zeolitic imidazolate framework-8 (AlgMA/CCM@ZIF-8) nanoparticles, which effectively eliminates ROS in BMSCs and simultaneously inhibits osteoclast differentiation. In addition to these direct effects on osteoblasts or osteoclasts, bone nanobiomaterials with the ability to regulate the immune microenvironment have become a research focus. The mechanisms can be mainly classified into three main categories (Fig. 3): first, the inherent characteristics of the biomaterials, particularly their surface physicochemical properties; second, the release of relevant ions in the material; and third, the construction of nano drug delivery systems with small molecule drugs. Mesoporous silica nanoparticles (MSNs) with an adjustable pore size (2–50 nm) are frequently used in bone regeneration because of their good biocompatibility, degradability, and drug-carrying properties (Fu et al., 2021; Hosseinpour et al., 2022). The

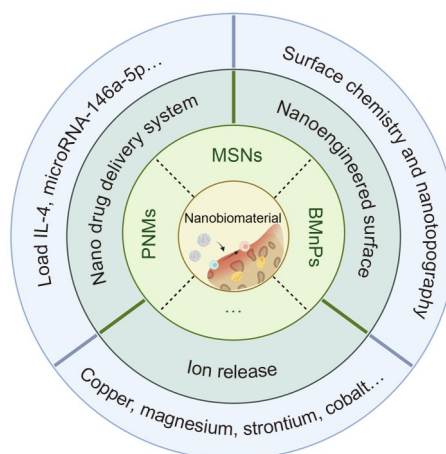


Fig. 3 Mechanisms of action of nanomaterials. Commonly used nanomaterials include mesoporous silica nanoparticles (MSNs), porous nanomaterials (PNMs), and bone mimetic nano hydroxyapatite particles (BMnPs). They play mainly an immunomodulatory role in bone regeneration: first, via their nanoengineered surface, including surface chemistry and nanotopography; second, by releasing associated ions such as copper, magnesium, strontium, and cobalt; and third, through their use as a nano drug-carrying system to release relevant small molecule drugs and directly act on immune cells. IL-4: interleukin-4. Created with BioRender.com.

immunomodulatory actions of MSNs can be divided into two main aspects: the activation of immune cells and the stimulation of cytokine release (Hosseinpour et al., 2022). According to previous reports, MSNs with appropriate shapes can inhibit the polarization of M1 macrophages and promote the polarization of M2 macrophages (Chen et al., 2018). This may be associated with the activation of TLRs or the release of ions in MSNs. For example, copper-doped MSNs (Cu-MSNs) can exert immunomodulatory effects, including enhanced expression of the C-C chemokine receptor 7 (CCR7) and complement receptor 4 (CR4, also known as CD11c) markers, and promote osteogenic differentiation of BMSCs by activating the oncostatin M (OSM) pathway (Shi et al., 2016). MSNs can also increase the secretion and release of IL-4 and IL-10 from Th1 and Th2 (Wang et al., 2016). The interaction between nanomaterials and the immune system is also related to their various characteristics, including size, surface charge, and surface morphology (Hosseinpour et al., 2022). Compared with MSNs featuring smooth surface, MSNs with cone-shaped pores on the surface (pore size 20 nm) can inhibit the secretion of inflammatory cytokines such as IL-1 β , IL-6, and TNF- α from macrophages (Xu et al., 2020).

Nanotopography, which refers to the surface roughness of nanomaterials, can modulate osteoblast activity, enhance osteogenic differentiation, and create an immune microenvironment favorable for bone regeneration (Chen et al., 2017b). Sadowska et al. (2018) reported that needle nanostructures of bionic calcium deficient hydroxyapatite (CDHA) promoted osteoblast differentiation better than sheet nanostructures did, whereas their needle nano surface morphology suppressed the expression of the pro-inflammatory factor TNF- α in RAW264.7 cells and regulated the immune microenvironment (Sadowska et al., 2018).

Because their polarization state significantly affects the timely abatement of inflammation in normal bone tissue regeneration, macrophages have become a major target for drug-loaded bone nano biomaterials (Schlundt et al., 2018). Chen et al. (2017a) reported that regulatory nanopore structures of different sizes can create distinct immune microenvironments, thereby altering macrophage morphology, inducing bone immune responses, and ultimately promoting the recruitment and differentiation of osteoblastic cell lineages. Their study revealed that, compared to smaller nanopores (20 nm), larger nanopores (200 nm) resulted in reduced macrophage adhesion but led to a higher degree of macrophage activation, with increased secretion of pro-inflammatory cytokines (Chen et al., 2017a). Kwon et al. (2017) reported that compared to traditional small pore nanoparticles, nanoparticles with a porosity of 30 nm exhibit superior IL-4 loading capacity, thereby promoting the M2 polarization of macrophages and attenuating local chronic inflammation. Yang et al. (2024) used magnesium silicate nanospheres (MSNs) loaded with microRNA-146a-5p (miR-146a-5p) to prepare a nanobiomaterial MSN+miR-146a, which was found to inhibit the NF- κ B pathway by targeting tumor necrosis factor receptor-associated factor 6 (TRAF6), while promoting M2 polarization of mouse BMMs. Mahon et al. (2020) invented an immunomodulatory scaffold containing bone mimetic nano hydroxyapatite particles (BMnPs), which can induce more macrophages into the M2 macrophages and upregulate the expression level of the anti-inflammatory factor IL-10. Negi et al. (2024) created a new method to accelerate bone healing that involves the use of acemannan-coated, cobalt-doped biphasic calcium phosphate nanoparticles. This material exhibited extreme stability and flexibility, and an ideal resorption rate, synergistically combining the roles of acetylated mannan and cobalt ions in tissue

regeneration. The results showed that the material could encourage macrophage polarization toward the M2 phenotype, mainly through the effect of cobalt on the induction of the M2 phenotype and the targeting of the phosphatidylinositol 3-kinase/protein kinase B/glycogen synthase kinase-3 β (PI3K/Akt/GSK-3 β) signaling pathway by acetylmannan (Ignjatović et al., 2013; Bai et al., 2023; Negi et al., 2024).

4.3 Challenges and future prospects for clinical application

Numerous nanomaterials for bone regeneration that exhibit immunomodulatory properties have been developed. However, the translation of these materials into clinical applications remains limited for several reasons. First, one reason is the significant disparity between the animal models used in research and real patients. In particular, the metabolic processes of small-molecule drugs loaded onto biomaterials in the human body remain unclear. Moreover, individual immune responses to the same material vary and are further influenced by disease conditions, increasing the demands on the precision of immune regulation in biomaterials. Second, the fabrication processes of these materials are highly complex, making large-scale production challenging and costly. Biomaterials intended for clinical applications must ensure sterility and safety during mass production, necessitating further advancements in relevant technologies and regulatory frameworks. Third, the current clinical evaluation system for bone implant materials focuses mainly on biocompatibility and mechanical properties, while there is no standardized assessment framework for their immunomodulatory functions. Additionally, ethical review processes and regulatory policies remain inadequate, further limiting the clinical translation of these materials.

Therefore, the development of immunomodulatory nanobiomaterials for bone regeneration with well-defined therapeutic targets, excellent biosafety, clear metabolic pathways, and ease of production and storage is key to accelerating research translation and ultimately achieving clinical application. Additionally, nearly all biomaterials cause an FBR after implantation, which creates irritation and causes an additional inflammatory response (Anderson et al., 2008; Newman et al., 2021). Hence, minimizing the FBRs of bone biomaterials and promoting their safe degradation of the materials are also major development goals.

5 Expectations

The immune microenvironment plays an important role in bone tissue regeneration and regulating the immune microenvironment serves as a key strategy for improving bone healing following inflammation. Nonetheless, additional research is needed to elucidate the complex crosstalk among diverse immune cells, osteoblasts, and osteoclasts, along with the temporal dynamics of cytokine networks during bone regeneration. In-depth exploration of key therapeutic targets in the immune microenvironment of bone regeneration will help us to better measure the level of inflammation at the bone defect site. The development of time-responsive and stimulus-sensitive immunomodulatory nanobiomaterials that enable the regulation of immune cell function under pathological conditions may maintain tissue homeostasis and create a favorable immune microenvironment for bone regeneration.

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Author contributions

Rong'an LI wrote the manuscript and created all the figures. Jiakang YANG conceived the study, supervised the research, and revised the final manuscript. Wenjun DUAN, Kexin YANG, and Katong LO provided the proofreading of the article. Qianming CHEN and Baixiang WANG hosted and managed the project. All authors have read and approved the final manuscript, and therefore, agreed to be responsible for the research integrity and for all aspects of the work.

Compliance with ethics guidelines

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This article does not contain any studies with human or animal subjects performed by any of the authors.

Declaration on the use of generative AI tools

During the preparation of this work, the authors used ChatGPT in order to improve language and readability, and

to check for grammatical errors. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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