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Review

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

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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). About 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).

Based on 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

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fixation. In the case of fractures in flat bones, such as the skull, direct healing takes place wherein progenitor cells directly undergo osteoblastic differentiation, leading to intramembranous ossification and subsequent formation of new bone matrix (Frost, 1989). Indirect healing, the more prevailing form of bone regeneration, is observed in most cases, encompassing both intramembranous and endochondral osteogenesis (Saul and Khosla, 2022). Irrespective of 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 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 and inflammatory cells, inflammatory factors, along with 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 interactions among the cells and molecules involved in bone regeneration under both physiological and pathological conditions remain incompletely understood.

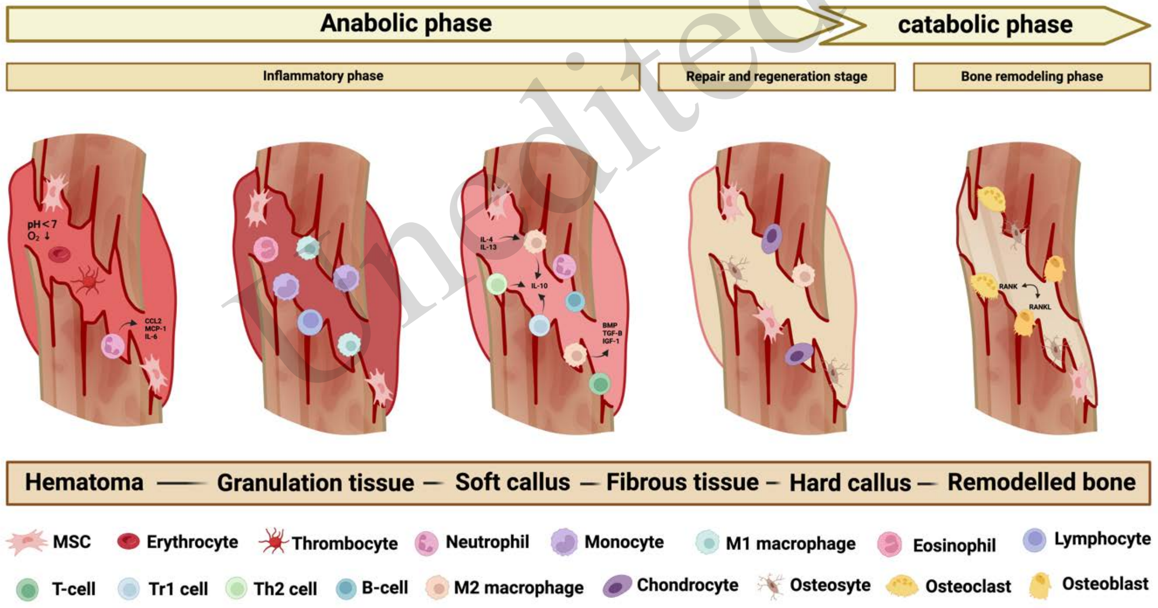

Fig. 1 The stages of bone healing.

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 cartilage and hard callus formation, culminating in new bone generation. Immune cells and cytokines are key regulators in this regenerative process. MSC: mesenchymal stem cells; Tr1 cell: type 1 regulatory T cells; Th2 cell: helper T cell 2. Created with BioRender.com.

The immune system, comprising 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. 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). Implementing 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 and development of bone nanobiomaterials with immune regulatory capabilities in 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 has been 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; Kolar et al., 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 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.

About 24-48 h after bone injury, neutrophils are replaced by monocytes, which differentiate into classically activated macrophages (CAM), also known as M1 pro-inflammatory 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 exhibit high levels of pro-inflammatory cytokine expression and release, mainly including interleukin-1 (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), C-X-C motif chemokine ligand 2 (CXCL2), and chemokine ligand 5 (CCL5) to further recruit lymphocytes and granulocytes (Arango Duque and Descoteaux, 2014; Huang et al., 2017). Effector T cells, including helper T cell 1 (Th1) and helper T cell 17 (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-κB 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 interaction. 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, 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-type 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. So 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 (AAM), namely M2 anti-inflammatory macrophages, and can induce CD4$^+$ T cells to produce interleukin-4 (IL-4), polarizing them into helper T cell 2(Th2), or differentiate into type 1 regulatory T cells (Tr1 cells) to secrete interleukin-10 (IL-10) (Jin et al., 2013; Fernando et al., 2014). Research has indicated that M2 macrophages, regulatory T-cells (Tregs), and Th2 cells are involved mainly in the phase of inflammation resolution (Schlundt et al., 2018; Schlundt et al., 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., 2017b). The presence of M1 macrophages in the early stages aids in pathogen clearance, activation of antimicrobial activity, and the induction of initial angiogenesis. However, prolonged M1 dominance can lead to chronic inflammation, ultimately inhibiting osteogenesis. Timely polarization toward the M2 phenotype supports tissue repair, whereas premature M2 polarization may reduce bone mineralization levels. Notably, the M1- and M2-dominated phases of tissue healing are not entirely independent. Therefore, in pathological conditions, precise regulation of the M1/M2 polarization time window is crucial for promoting bone regeneration.

As the main group of macrophages 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). Besides IL-10, the anti-inflammatory factors IL-4 and IL-13 released by CD4$^+$ T cells, granulocytes, and NK cells cooperate with IL-10 to stabilize the polarization of macrophages into M2 type, and promote the osteogenic differentiation and mineralization of bone marrow stromal cell (BMSC) (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 differentiate and proliferate 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 a crucial positive regulatory role 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 concludes with the apoptosis of chondrocytes. and 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).

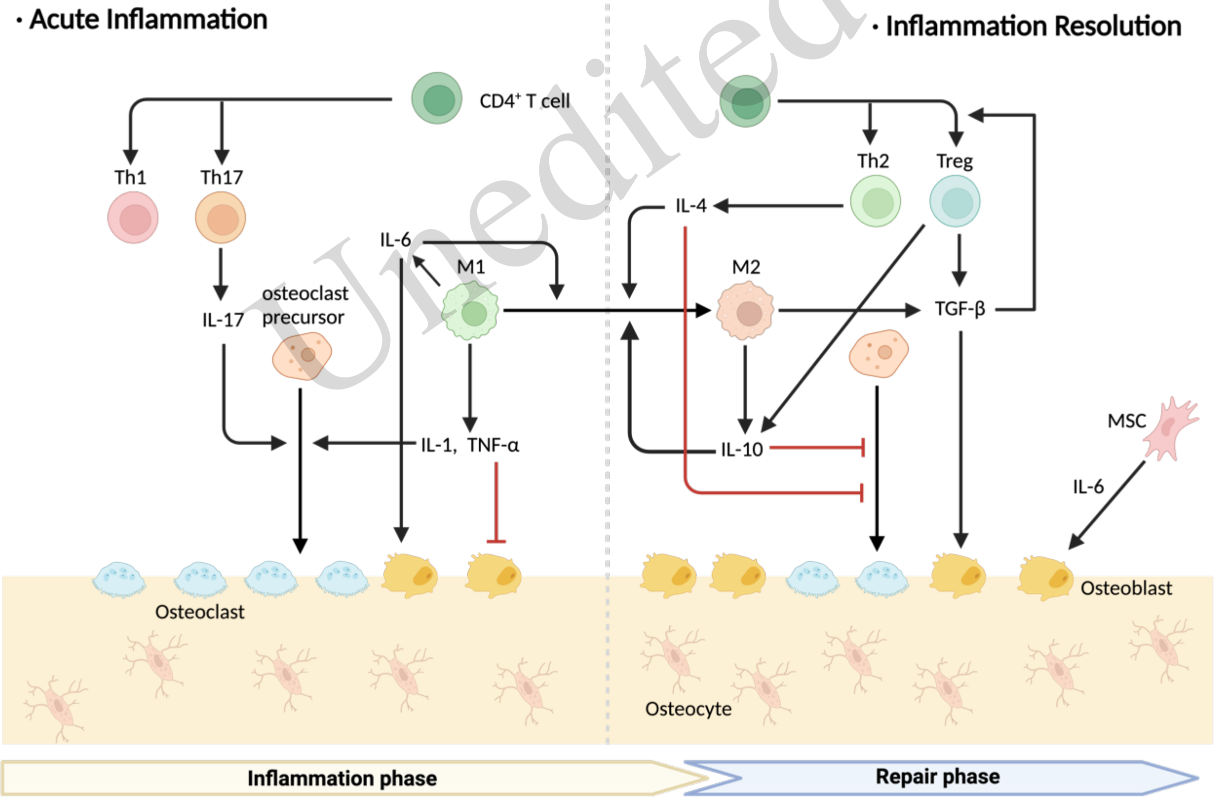

Fig. 2 Interactions between the immune and skeletal systems in bone regeneration
The diagram shows how immune cells regulate the balance between inflammation and bone metabolism in a temporal manner. In the early stage of inflammation, Th1, Th17 and M1 macrophages are activated and release pro-inflammatory factors such as IL-1 and 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, Treg 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. Created with BioRender.com.

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 some common characteristics, including substantial upregulation of pro-inflammatory factors like 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 to treat inflammatory bone defects.

3.1 Effect of excessive acute inflammation on bone regeneration

Studies have shown that excessive acute inflammation, 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 their overaccumulation at the bone defect site, which in turn impairs bone healing (Bastian et al., 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 time of maturation of fracture callus 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 into osteoclasts, and suppress the mRNA expression of RUNX2, Osterix (OSX), and osteocalcin (OCN) to inhibit osteoblast differentiation (Hoff et al., 2017; Takeuchi, et al., 2021). $CD4^+$ 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-type 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 nuclear factor κB receptor activator (RANK) on the surface of osteoclasts to activate the nuclear transcription factor-κB (NF-κB) pathway and induce osteoclast differentiation and activation (Hardy and Cooper, 2009; Claes, et al., 2012; Lim, et al., 2017). The NF-κB 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). Increased in pro-inflammatory cytokines negatively affect bone regeneration directly. 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, like periodontitis, diabetes, rheumatoid arthritis 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 kind of inflammatory disease associated with microecological imbalance (Jacobs et al., 2024). It is related to the plaque biofilm 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 osteoclast activity in the alveolar bone is significantly enhanced, 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 NF-κB 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 source of RANKL in periodontitis (Mantovani et al., 2011; Sadek, et al., 2023). Both TNF-α and IL-1 downregulate 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, 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 higher than 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 will be discussed below.

Patients with diabetes also exhibit weak bone healing and delayed fracture repair, with a higher 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 upregulation 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 vascular endothelial growth factor (VEGF) is observed in the bone regeneration area, and the dysregulated 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-1β, IL-6 and IL-18, are also significantly elevated, further activating the NF-κB pathway, amplifying inflammation, and delaying its resolution (Jiao, et al., 2015; Ko et al., 2019). Research has shown that excessive NF-κB pathway activation decreases TGF-β1 expression in endogenous skeletal stem cells (SSCs), causing defective polarization and dysfunction of macrophages. However, blocking the NF-κB pathway or intervention with exogenous TGF-β1 can rescue bone regeneration within a high-glucose microenvironment (Ko, et al., 2019).

Physiological ageing 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 down-regulated 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 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 senescence-associated secretory phenotype (SASP) (Josephson et al., 2019). 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-κB signaling pathway (Salminen et al., 2012; Goodnough and Goodman, 2022). As a key driver of the SASP, NF-κB 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 a 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 6 weeks post-fracture, promoting tissue vascularization and reducing fibrosis (Loffler 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) in 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 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-κB 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 The characteristics and advantages of nanobiomaterials

Biomaterials have been widely used in bone tissue engineering, not only providing spatial support for regeneration, but also serving as carriers for the delivery of drugs, growth factors, genes and cells (Lee et al., 2019). According to the size of their structural morphology, bone biomaterials can be divided into nano-, micro- and macro-materials (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. As drug carriers, due to their nanoscale size, they can be efficiently internalized by cells through endocytosis (Gulati et al., 2022). Compared to micrometer-size materials, nanomaterials have increased specific surface area, better biodegradability and biological activity, and more bioactive sites (Bramhill et al., 2017). For example, 10 wt% 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, increase 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 (FBGC) 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 failing to meet the needs of complex clinical bone defect cases, especially in patients with associated diseases including diabetes, rheumatoid arthritis 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 by the material itself or the specific drug molecules it is carrying (Newman, et al., 2021; Lopez, et al., 2022). Qiao et al. (2021) found that in a rat femoral defect model, implantation of alginate saline gel containing Mg^{2+} induced the production of multinucleated giant cells (MNGCs), which highly expressed 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 express M2 macrophage-like wound healing and inflammation-terminating molecules, such as Ym1, Arg1, and 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; Veiseh and Vegas, 2019).

4.2 The pathways of action of immunomodulatory bone nanobiomaterials

Apart from directly acting 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 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 due to their good biocompatibility, degradability and drug-carrying properties (Fu et al., 2021; Hosseinpour et al., 2022). The 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 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 toll-like receptors (TLRs) or the release of ions in MSNs. For example, copper-doped mesoporous silica nanospheres (Cu-MSNs) can exert immunomodulatory effects, including enhanced expression of the CCR7 and CD11c markers, and promote osteogenic differentiation of BMSCs by activating the oncostatin M (OSM) pathway (Shi et al., 2016). MSNs also can boost 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 to MSNs with a smooth surface, MSNs with cone-shaped pores on the surface (pore size 20 nm) can inhibit the secretion of inflammatory cytokines such as IL-1β, 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., 2017a). Sadowska et al. (2018) found that needle nanostructures of bionic calcium deficient hydroxyapatite (CDHA) promoted osteoblast differentiation better than sheet nanostructures, while its 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).

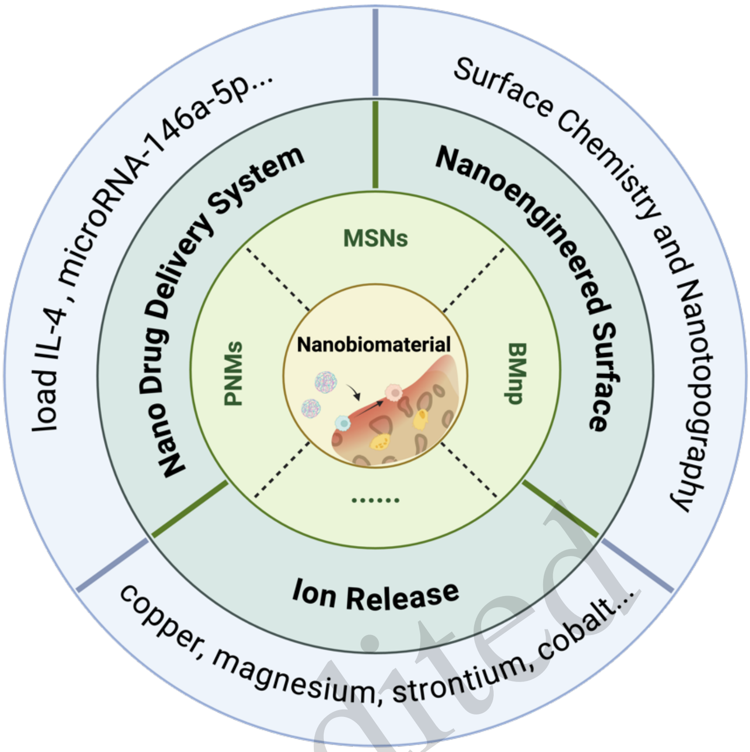

Fig. 3 Mechanisms of action of nanomaterials

Commonly used nanomaterials include mesoporous silica nanoparticles (MSNs), porous nanomaterials (PMNS), and bone mimetic nano hydroxyapatite particles (BMnPs). They play mainly an immunomodulatory role in bone regeneration, firstly via their nanoengineered surface, including surface chemistry and nanotopography, secondly, by releasing associated ions such as copper, magnesium, strontium, and cobalt, and thirdly through their use as a nano drug-carrying system to release relevant small molecule drugs and directly act on immune cells. Created with BioRender.com.

Because their polarization state significantly affects the timely abatement of inflammation in normal bone tissue regeneration, microphages have become a major target for drug-loaded bone nano biomaterials (Schlundt, et al., 2018). Chen et al. (2017) found 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., 2017b). Kwon et al. (2017) found that nanoparticles with a porosity of 30 nm exhibit superior IL-4 loading capacity compared to traditional small pore nanoparticles, thereby promoting the M2 polarization of macrophages and attenuating local chronic inflammation (Kwon, et al., 2017). Yang et al. (2024) used magnesium silicate nanospheres (MSNs) loaded with microRNA-146a-5p (miR-146a) to prepare a nanobiomaterial MSN+miR-146a, which was found to inhibit the NF-κB pathway by targeting tumor necrosis factor receptor associated factor 6 (TRAF6), while promoting M2 polarization of mouse bone marrow-derived macrophages (BMMs) (Yang et al., 2024). Mahon et al. (2020) invented an immunomodulatory scaffold containing bone mimetic nano hydroxyapatite particles (BMnPs), which can induce more macrophages into the M2-type and up-regulate the expression level of the anti-inflammatory factor IL-10 (Mahon, et al., 2020). 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 (Negi et al., 2024). 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 towards the M2 phenotype, mainly through the effect of cobalt on the induction of the M2 phenotype and the targeting of the PI3K/Akt/GSK-3β signaling pathway by acetylmannan (Ignjatovic 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 exhibiting immunomodulatory properties have been developed. However, the translation of these materials into clinical applications remains limited for several reasons. 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, raising higher demands on the precision of immune regulation in biomaterials. Secondly, 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. Thirdly, 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 foreign body reaction of bone biomaterials and promoting the safe degradation of the materials is also a major development goal.

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 required 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 detect the level of inflammation at the bone defect site. Developing time-responsive and stimulus-sensitive immunomodulatory nanobiomaterials which 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

These authors contributed equally: Rongan LI, Jiakang YANG.

Rongan LI wrote the manuscript and completed the drawing of all the figures. Jiakang YANG designed the conception, supervised, 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 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

Rongan LI, Jiakang YANG, Wenjun DUAN, Kexin YANG, Katong LO, Qianming CHEN and Baixiang WANG declare that they have no conflict of interest.

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

References

Alblowi J, Kayal RA, Siqueira M, et al., 2009. High levels of tumor necrosis factor-alpha contribute to accelerated loss of cartilage in diabetic fracture healing. *Am J Pathol*, 175(4):1574-1585. https://doi.org/10.2353/ajpath.2009.090148

Anderson JM, Rodriguez A, Chang DT, 2008. Foreign body reaction to biomaterials. *Semin Immunol*, 20(2):86-100. https://doi.org/10.1016/j.smim.2007.11.004

Arango Duque G, Descoteaux A, 2014. Macrophage cytokines: Involvement in immunity and infectious diseases. *Front Immunol*, 5:491. https://doi.org/10.3389/fimmu.2014.00491

Bahney CS, Zondervan RL, Allison P, et al., 2019. Cellular biology of fracture healing. *J Orthop Res*, 37(1):35-50. https://doi.org/10.1002/jor.24170

Bai Y, Niu Y, Qin S, et al., 2023. A new biomaterial derived from aloe vera-acemannan from basic studies to clinical application. *Pharmaceutics*, 15(7) https://doi.org/10.3390/pharmaceutics15071913

Balistreri CR, Candore G, Accardi G, et al., 2013. Nf-kappab pathway activators as potential ageing biomarkers: Targets for new therapeutic strategies. *Immun Ageing*, 10(1):24. https://doi.org/10.1186/1742-4933-10-24

Bastian O, Pillay J, Alblas J, et al., 2011. Systemic inflammation and fracture healing. *J Leukoc Biol*, 89(5):669-673. https://doi.org/10.1189/jlb.0810446

Bramhill J, Ross S, Ross G, 2017. Bioactive nanocomposites for tissue repair and regeneration: A review. *Int J Environ Res Public Health*, 14(1) https://doi.org/10.3390/ijerph14010066

Cao L, Werkmeister JA, Wang J, et al., 2014. Bone regeneration using photocrosslinked hydrogel incorporating rhbmp-2 loaded 2-n, 6-o-sulfated chitosan nanoparticles. *Biomaterials*, 35(9):2730-2742. https://doi.org/10.1016/j.biomaterials.2013.12.028

Chen Z, Bachhuka A, Han S, et al., 2017a. Tuning chemistry and topography of nanoengineered surfaces to manipulate immune response for bone regeneration applications. *ACS Nano*, 11(5):4494-4506. https://doi.org/10.1021/acsnano.6b07808

Chen Z, Bachhuka A, Wei F, et al., 2017b. Nanotopography-based strategy for the precise manipulation of osteoimmunomodulation in bone regeneration. *Nanoscale*, 9(46):18129-18152. https://doi.org/10.1039/c7nr05913b

Chen ZT, Han SW, Shi MC, et al., 2018. Immunomodulatory effects of mesoporous silica nanoparticles on osteogenesis: From nanoimmunotoxicity to nanoimmunotherapy. *Applied Materials Today*, 10:184-193. https://doi.org/10.1016/j.apmt.2017.12.003

Chung R, Cool JC, Scherer MA, et al., 2006. Roles of neutrophil-mediated inflammatory response in the bony repair of injured growth plate cartilage in young rats. *J Leukoc Biol*, 80(6):1272-1280. https://doi.org/10.1189/jlb.0606365

Claes L, Recknagel S, Ignatius A, 2012. Fracture healing under healthy and inflammatory conditions. *Nat Rev Rheumatol*, 8(3):133-143. https://doi.org/10.1038/nrrheum.2012.1

Cottrell J, O'connor JP, 2010. Effect of non-steroidal anti-inflammatory drugs on bone healing. *Pharmaceuticals (Basel)*, 3(5):1668-1693. https://doi.org/10.3390/ph3051668

Davison NL, Su J, Yuan H, et al., 2015. Influence of surface microstructure and chemistry on osteoinduction and osteoclastogenesis by biphasic calcium phosphate discs. *Eur Cell Mater*, 29:314-329. https://doi.org/10.22203/ecm.v029a24

Ding ZC, Zeng WN, Rong X, et al., 2020. Do patients with diabetes have an increased risk of impaired fracture healing? A systematic review and meta-analysis. *ANZ J Surg*, 90(7-8):1259-1264. https://doi.org/10.1111/ans.15878

Donath MY, Shoelson SE, 2011. Type 2 diabetes as an inflammatory disease. *Nat Rev Immunol*, 11(2):98-107. https://doi.org/10.1038/nri2925

Duda GN, Geissler S, Checa S, et al., 2023. The decisive early phase of bone regeneration. *Nat Rev Rheumatol*, 19(2):78-95. https://doi.org/10.1038/s41584-022-00887-0

Einhorn TA, Gerstenfeld LC, 2015. Fracture healing: Mechanisms and interventions. *Nat Rev Rheumatol*, 11(1):45-54. https://doi.org/10.1038/nrrheum.2014.164

Ellis TN, Beaman BL, 2004. Interferon-gamma activation of polymorphonuclear neutrophil function. *Immunology*, 112(1):2-12. https://doi.org/10.1111/j.1365-2567.2004.01849.x

Fernando MR, Reyes JL, Iannuzzi J, et al., 2014. The pro-inflammatory cytokine, interleukin-6, enhances the polarization of alternatively activated macrophages. *PLoS One*, 9(4):e94188. https://doi.org/10.1371/journal.pone.0094188

Ferrari SL, Abrahamsen B, Napoli N, et al., 2018. Diagnosis and management of bone fragility in diabetes: An emerging challenge. *Osteoporos Int*, 29(12):2585-2596. https://doi.org/10.1007/s00198-018-4650-2

Fijany A, Sayadi LR, Khoshab N, et al., 2019. Mesenchymal stem cell dysfunction in diabetes. *Mol Biol Rep*, 46(1):1459-1475. https://doi.org/10.1007/s11033-018-4516-x

Franceschi C, Bonafe M, Valensin S, et al., 2000. Inflamm-aging. An evolutionary perspective on immunosenescence. *Ann N Y Acad Sci*, 908:244-254. https://doi.org/10.1111/j.1749-6632.2000.tb06651.x

Fratzl P, Gupta HS, Paschalis EP, et al., 2004. Structure and mechanical quality of the collagen-mineral nano-composite in bone.

Journal of Materials Chemistry, 14(14):2115-2123. https://doi.org/10.1039/b402005g

Frost HM, 1989. The biology of fracture healing. An overview for clinicians. Part ii. *Clin Orthop Relat Res*, (248):294-309.

Fu X, Han B, Cai S, et al., 2009. Migration of bone marrow-derived mesenchymal stem cells induced by tumor necrosis factor-alpha and its possible role in wound healing. *Wound Repair Regen*, 17(2):185-191. https://doi.org/10.1111/j.1524-475X.2009.00454.x

Fu Y, Cui S, Luo D, et al., 2021. Novel inorganic nanomaterial-based therapy for bone tissue regeneration. *Nanomaterials (Basel)*, 11(3) https://doi.org/10.3390/nano11030789

Gerstenfeld LC, Cho TJ, Kon T, et al., 2003. Impaired fracture healing in the absence of tnf-alpha signaling: The role of tnf-alpha in endochondral cartilage resorption. *J Bone Miner Res*, 18(9):1584-1592. https://doi.org/10.1359/jbmr.2003.18.9.1584

Goodnough LH, Goodman SB, 2022. Relationship of aging, inflammation, and skeletal stem cells and their effects on fracture repair. *Curr Osteoporos Rep*, 20(5):320-325. https://doi.org/10.1007/s11914-022-00742-x

Gulati K, Abdal-Hay A, Ivanovski S, 2022. Novel nano-engineered biomaterials for bone tissue engineering. *Nanomaterials (Basel)*, 12(3) https://doi.org/10.3390/nano12030333

Hardy R, Cooper MS, 2009. Bone loss in inflammatory disorders. *J Endocrinol*, 201(3):309-320. https://doi.org/10.1677/JOE-08-0568

Hoff P, Gaber T, Strehl C, et al., 2017. A pronounced inflammatory activity characterizes the early fracture healing phase in immunologically restricted patients. *Int J Mol Sci*, 18(3) https://doi.org/10.3390/ijms18030583

Hosseinpour S, Walsh LJ, Xu C, 2022. Modulating osteoimmune responses by mesoporous silica nanoparticles. *ACS Biomater Sci Eng*, 8(10):4110-4122. https://doi.org/10.1021/acsbiomaterials.1c00899

Hozain S, Cottrell J, 2020. Cdllb+ targeted depletion of macrophages negatively affects bone fracture healing. *Bone*, 138:115479. https://doi.org/10.1016/j.bone.2020.115479

Huang R, Wang X, Zhou Y, et al., 2017. Rankl-induced m1 macrophages are involved in bone formation. *Bone Res*, 5:17019. https://doi.org/10.1038/boneres.2017.19

Huang RL, Sun Y, Ho CK, et al., 2018. Il-6 potentiates bmp-2-induced osteogenesis and adipogenesis via two different bmpr1a-mediated pathways. *Cell Death Dis*, 9(2):144. https://doi.org/10.1038/s41419-017-0126-0

Huang X, Xie M, Xie Y, et al., 2020. The roles of osteocytes in alveolar bone destruction in periodontitis. *J Transl Med*, 18(1):479. https://doi.org/10.1186/s12967-020-02664-7

Hurtgen BJ, Ward CL, Garg K, et al., 2016. Severe muscle trauma triggers heightened and prolonged local musculoskeletal inflammation and impairs adjacent tibia fracture healing. *J Musculoskelet Neuronal Interact*, 16(2):122-134.

Ignjatovic N, Ajdukovic Z, Savic V, et al., 2013. Nanoparticles of cobalt-substituted hydroxyapatite in regeneration of mandibular osteoporotic bones. *J Mater Sci Mater Med*, 24(2):343-354. https://doi.org/10.1007/s10856-012-4793-1

Jacobs R, Fontenele RC, Lahoud P, et al., 2024. Radiographic diagnosis of periodontal diseases - current evidence versus innovations. *Periodontol 2000*, 95(1):51-69. https://doi.org/10.1111/prd.12580

Jiao H, Xiao E, Graves DT, 2015. Diabetes and its effect on bone and fracture healing. *Curr Osteoporos Rep*, 13(5):327-335. https://doi.org/10.1007/s11914-015-0286-8

Jimi E, Fei H, Nakatomi C, 2019. Nf-kappab signaling regulates physiological and pathological chondrogenesis. *Int J Mol Sci*, 20(24) https://doi.org/10.3390/ijms20246275

Jin JO, Han X, Yu Q, 2013. Interleukin-6 induces the generation of il-10-producing tr1 cells and suppresses autoimmune tissue inflammation. *J Autoimmun*, 40:28-44. https://doi.org/10.1016/j.jaut.2012.07.009

Josephson AM, Bradaschia-Correa V, Lee S, et al., 2019. Age-related inflammation triggers skeletal stem/progenitor cell dysfunction. *Proc Natl Acad Sci U S A*, 116(14):6995-7004. https://doi.org/10.1073/pnas.1810692116

Julier Z, Park AJ, Briquez PS, et al., 2017. Promoting tissue regeneration by modulating the immune system. *Acta Biomater*, 53:13-28. https://doi.org/10.1016/j.actbio.2017.01.056

Katsuyama E, Miyamoto H, Kobayashi T, et al., 2015. Interleukin-1 receptor-associated kinase-4 (irak4) promotes inflammatory osteolysis by activating osteoclasts and inhibiting formation of foreign body giant cells. *J Biol Chem*, 290(2):716-726. https://doi.org/10.1074/jbc.M114.568360

Ko KI, Coimbra LS, Tian C, et al., 2015. Diabetes reduces mesenchymal stem cells in fracture healing through a tnfalpha-mediated mechanism. *Diabetologia*, 58(3):633-642. https://doi.org/10.1007/s00125-014-3470-y

Ko KI, Syverson AL, Kralik RM, et al., 2019. Diabetes-induced nf-kappab dysregulation in skeletal stem cells prevents resolution of inflammation. *Diabetes*, 68(11):2095-2106. https://doi.org/10.2337/db19-0496

Kobayashi K, Takahashi N, Jimi E, et al., 2000. Tumor necrosis factor alpha stimulates osteoclast differentiation by a mechanism independent of the odf/rankl-rank interaction. *J Exp Med*, 191(2):275-286. https://doi.org/10.1084/jem.191.2.275

Kolar P, Schmidt-Bleek K, Schell H, et al., 2010. The early fracture hematoma and its potential role in fracture healing. *Tissue Eng Part B Rev*, 16(4):427-434. https://doi.org/10.1089/ten.TEB.2009.0687

Kolar P, Gaber T, Perka C, et al., 2011. Human early fracture hematoma is characterized by inflammation and hypoxia. *Clin Orthop Relat Res*, 469(11):3118-3126. https://doi.org/10.1007/s11999-011-1865-3

Kovtun A, Bergdolt S, Wiegner R, et al., 2016. The crucial role of neutrophil granulocytes in bone fracture healing. *Eur Cell Mater*, 32:152-162. https://doi.org/10.22203/ecm.v032a10

Kuchler U, Schwarze UY, Dobsak T, et al., 2014. Dental and periodontal phenotype in sclerostin knockout mice. *Int J Oral Sci*, 6(2):70-76. https://doi.org/10.1038/ijos.2014.12

Kushioka J, Chow SK, Toya M, et al., 2023. Bone regeneration in inflammation with aging and cell-based immunomodulatory therapy. *Inflamm Regen*, 43(1):29. https://doi.org/10.1186/s41232-023-00279-1

Kwon D, Cha BG, Cho Y, et al., 2017. Extra-large pore mesoporous silica nanoparticles for directing in vivo m2 macrophage polarization by delivering il-4. *Nano Lett*, 17(5):2747-2756. https://doi.org/10.1021/acs.nanolett.6b04130

Lee J, Byun H, Madhurakkat Perikamana SK, et al., 2019. Current advances in immunomodulatory biomaterials for bone regeneration. *Adv Healthc Mater*, 8(4):e1801106. https://doi.org/10.1002/adhm.201801106

Lei H, Schmidt-Bleek K, Dienelt A, et al., 2015. Regulatory t cell-mediated anti-inflammatory effects promote successful tissue repair in both indirect and direct manners. *Front Pharmacol*, 6:184. https://doi.org/10.3389/fphar.2015.00184

Lim JC, Ko KI, Mattos M, et al., 2017. Tnfalpha contributes to diabetes impaired angiogenesis in fracture healing. *Bone*, 99:26-38. https://doi.org/10.1016/j.bone.2017.02.014

Liu D, Xu JK, Figliomeni L, et al., 2003. Expression of rankl and opg mrna in periodontal disease: Possible involvement in bone destruction. *Int J Mol Med*, 11(1):17-21. https://doi.org/10.3892/ijmm.11.1.17

Loffler J, Sass FA, Filter S, et al., 2019. Compromised bone healing in aged rats is associated with impaired m2 macrophage function. *Front Immunol*, 10:2443. https://doi.org/10.3389/fimmu.2019.02443

Loi F, Cordova LA, Pajarinen J, et al., 2016. Inflammation, fracture and bone repair. *Bone*, 86:119-130. https://doi.org/10.1016/j.bone.2016.02.020

Lopez EM, Leclerc K, Ramsukh M, et al., 2022. Modulating the systemic and local adaptive immune response after fracture improves bone regeneration during aging. *Bone*, 157:116324. https://doi.org/10.1016/j.bone.2021.116324

Mahon OR, Browe DC, Gonzalez-Fernandez T, et al., 2020. Nano-particle mediated m2 macrophage polarization enhances bone formation and msc osteogenesis in an il-10 dependent manner. *Biomaterials*, 239:119833. https://doi.org/10.1016/j.biomaterials.2020.119833

Mantovani A, Cassatella MA, Costantini C, et al., 2011. Neutrophils in the activation and regulation of innate and adaptive immunity. *Nat Rev Immunol*, 11(8):519-531. https://doi.org/10.1038/nri3024

Marsell R, Einhorn TA, 2011. The biology of fracture healing. *Injury*, 42(6):551-555. https://doi.org/10.1016/j.injury.2011.03.031

Martino MM, Maruyama K, Kuhn GA, et al., 2016. Inhibition of il-1r1/myd88 signalling promotes mesenchymal stem cell-driven tissue regeneration. *Nat Commun*, 7:11051. https://doi.org/10.1038/ncomms11051

Maruyama M, Rhee C, Utsunomiya T, et al., 2020. Modulation of the inflammatory response and bone healing. *Front Endocrinol (Lausanne)*, 11:386. https://doi.org/10.3389/fendo.2020.00386

Mooney JE, Rolfe BE, Osborne GW, et al., 2010. Cellular plasticity of inflammatory myeloid cells in the peritoneal foreign body response. *Am J Pathol*, 176(1):369-380. https://doi.org/10.2353/ajpath.2010.090545

Negi D, Bhavya K, Pal D, et al., 2024. Acemannan coated, cobalt-doped biphasic calcium phosphate nanoparticles for immunomodulation regulated bone regeneration. *Biomater Sci*, 12(14):3672-3685. https://doi.org/10.1039/d4bm00482e

Newman H, Shih YV, Varghese S, 2021. Resolution of inflammation in bone regeneration: From understandings to therapeutic applications. *Biomaterials*, 277:121114. https://doi.org/10.1016/j.biomaterials.2021.121114

Oh SA, Li MO, 2013. Tgf-beta: Guardian of t cell function. *J Immunol*, 191(8):3973-3979. https://doi.org/10.4049/jimmunol.1301843

Olivieri F, Prattichizzo F, Grillari J, et al., 2018. Cellular senescence and inflammaging in age-related diseases. *Mediators Inflamm*, 2018:9076485. https://doi.org/10.1155/2018/9076485

Pacios S, Andriankaja O, Kang J, et al., 2013. Bacterial infection increases periodontal bone loss in diabetic rats through enhanced apoptosis. *Am J Pathol*, 183(6):1928-1935. https://doi.org/10.1016/j.ajpath.2013.08.017

Pajarinen J, Lin T, Gibon E, et al., 2019. Mesenchymal stem cell-macrophage crosstalk and bone healing. *Biomaterials*, 196:80-89. https://doi.org/10.1016/j.biomaterials.2017.12.025

Qiao W, Xie H, Fang J, et al., 2021. Sequential activation of heterogeneous macrophage phenotypes is essential for biomaterials-induced bone regeneration. *Biomaterials*, 276:121038. https://doi.org/10.1016/j.biomaterials.2021.121038

Relja B, Yang B, Bundkirchen K, et al., 2020. Different experimental multiple trauma models induce comparable inflammation and organ injury. *Sci Rep*, 10(1):20185. https://doi.org/10.1038/s41598-020-76499-z

Rezaei Esfahrood Z, Yadegari Z, Veysari SK, et al., 2018. Gingival crevicular fluid levels of sclerostin in chronic periodontitis and healthy subjects. *J Korean Assoc Oral Maxillofac Surg*, 44(6):289-292. https://doi.org/10.5125/jkaoms.2018.44.6.289

Sadek KM, El Moshy S, Radwan IA, et al., 2023. Molecular basis beyond interrelated bone resorption/regeneration in periodontal diseases: A concise review. *Int J Mol Sci*, 24(5) https://doi.org/10.3390/ijms24054599

Sadowska JM, Wei F, Guo J, et al., 2018. Effect of nano-structural properties of biomimetic hydroxyapatite on osteoimmunomodulation. *Biomaterials*, 181:318-332. https://doi.org/10.1016/j.biomaterials.2018.07.058

Salminen A, Kauppinen A, Kaarniranta K, 2012. Emerging role of nf-kappab signaling in the induction of senescence-associated secretory phenotype (sasp). *Cell Signal*, 24(4):835-845. https://doi.org/10.1016/j.cellsig.2011.12.006

Sasi SP, Yan X, Enderling H, et al., 2012. Breaking the 'harmony' of tnf-alpha signaling for cancer treatment. *Oncogene*, 31(37):4117-4127. https://doi.org/10.1038/onc.2011.567

Saul D, Khosla S, 2022. Fracture healing in the setting of endocrine diseases, aging, and cellular senescence. *Endocr Rev*, 43(6):984-1002. https://doi.org/10.1210/endrev/bnac008

Schlundt C, El Khassawna T, Serra A, et al., 2018. Macrophages in bone fracture healing: Their essential role in endochondral ossification. *Bone*, 106:78-89. https://doi.org/10.1016/j.bone.2015.10.019

Schlundt C, Reinke S, Geissler S, et al., 2019. Individual effector/regulator t cell ratios impact bone regeneration. *Front Immunol*, 10:1954. https://doi.org/10.3389/fimmu.2019.01954

Shemesh M, Addadi L, Geiger B, 2017. Surface microtopography modulates sealing zone development in osteoclasts cultured on bone. *J R Soc Interface*, 14(127) https://doi.org/10.1098/rsif.2016.0958

Shi M, Chen Z, Farnaghi S, et al., 2016. Copper-doped mesoporous silica nanospheres, a promising immunomodulatory agent for inducing osteogenesis. *Acta Biomater*, 30:334-344. https://doi.org/10.1016/j.actbio.2015.11.033

Shull MM, Ormsby I, Kier AB, et al., 1992. Targeted disruption of the mouse transforming growth factor-beta 1 gene results in multifocal inflammatory disease. *Nature*, 359(6397):693-699. https://doi.org/10.1038/359693a0

Takeuchi T, Yoshida H, Tanaka S, 2021. Role of interleukin-6 in bone destruction and bone repair in rheumatoid arthritis. *Autoimmun Rev*, 20(9):102884. https://doi.org/10.1016/j.autrev.2021.102884

Tanios M, Brickman B, Cage E, et al., 2022. Diabetes and impaired fracture healing: A narrative review of recent literature. *Curr Osteoporos Rep*, 20(5):229-239. https://doi.org/10.1007/s11914-022-00740-z

Timmen M, Hidding H, Wieskotter B, et al., 2014. Influence of antitnf-alpha antibody treatment on fracture healing under chronic inflammation. *BMC Musculoskelet Disord*, 15:184. https://doi.org/10.1186/1471-2474-15-184

Trindade R, Albrektsson T, Tengvall P, et al., 2016. Foreign body reaction to biomaterials: On mechanisms for buildup and breakdown of osseointegration. *Clin Implant Dent Relat Res*, 18(1):192-203. https://doi.org/10.1111/cid.12274

Veiseh O, Vegas AJ, 2019. Domesticating the foreign body response: Recent advances and applications. *Adv Drug Deliv Rev*, 144:148-161. https://doi.org/10.1016/j.addr.2019.08.010

Wang X, Li X, Yoshiyuki K, et al., 2016. Comprehensive mechanism analysis of mesoporous-silica-nanoparticle-induced cancer immunotherapy. *Adv Healthc Mater*, 5(10):1169-1176. https://doi.org/10.1002/adhm.201501013

Weckbach S, Hohmann C, Braumueller S, et al., 2013. Inflammatory and apoptotic alterations in serum and injured tissue after experimental polytrauma in mice: Distinct early response compared with single trauma or "double-hit" injury. *J Trauma Acute Care Surg*, 74(2):489-498. https://doi.org/10.1097/TA.0b013e31827d5f1b

Weitzmann MN, 2017. Bone and the immune system. *Toxicol Pathol*, 45(7):911-924. https://doi.org/10.1177/0192623317735316

Xu C, Xiao L, Gao YX, et al., 2020. Mesoporous silica rods with cone shaped pores modulate inflammation and deliver bmp-2 for bone regeneration. *Nano Research*, 13(9):2323-2331. https://doi.org/10.1007/s12274-020-2783-z

Yang J, Shuai J, Siow L, et al., 2024. Microrna-146a-loaded magnesium silicate nanospheres promote bone regeneration in an inflammatory microenvironment. *Bone Res*, 12(1):2. https://doi.org/10.1038/s41413-023-00299-0

Yang N, Liu Y, 2021. The role of the immune microenvironment in bone regeneration. *Int J Med Sci*, 18(16):3697-3707. https://doi.org/10.7150/ijms.61080

Yang X, Ricciardi BF, Hernandez-Soria A, et al., 2007. Callus mineralization and maturation are delayed during fracture healing in interleukin-6 knockout mice. *Bone*, 41(6):928-936. https://doi.org/10.1016/j.bone.2007.07.022

Yuasa M, Mignemi NA, Nyman JS, et al., 2015. Fibrinolysis is essential for fracture repair and prevention of heterotopic ossification. *J Clin Invest*, 125(8):3117-3131. https://doi.org/10.1172/JCI80313

Zaiss MM, Frey B, Hess A, et al., 2010. Regulatory t cells protect from local and systemic bone destruction in arthritis. *J Immunol*, 184(12):7238-7246. https://doi.org/10.4049/jimmunol.0903841

Zhang E, Miramini S, Patel M, et al., 2022. Role of tnf-alpha in early-stage fracture healing under normal and diabetic conditions. *Comput Methods Programs Biomed*, 213:106536. https://doi.org/10.1016/j.cmpb.2021.106536

Zhang Q, Chen B, Yan F, et al., 2014. Interleukin-10 inhibits bone resorption: A potential therapeutic strategy in periodontitis and other bone loss diseases. *Biomed Res Int*, 2014:284836. https://doi.org/10.1155/2014/284836

Zheng XY, Mao CY, Qiao H, et al., 2017. Plumbagin suppresses chronic periodontitis in rats via down-regulation of tnf-alpha, il-1beta and il-6 expression. *Acta Pharmacol Sin*, 38(8):1150-1160. https://doi.org/10.1038/aps.2017.19