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Immune homeostasis modulation by hydrogel-guided delivery systems: a tool for accelerated bone regeneration

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Immune homeostasis is delicately mediated by the dynamic balance between effector immune cells and regulatory immune cells. Local deviations from immune homeostasis in the microenvironment of bone fractures, caused by an increased ratio of effector to regulatory cues, can lead to excessive inflammatory conditions and hinder bone regeneration. Therefore, achieving effective and localized immunomodulation of bone fractures is crucial for successful bone regeneration. Recent research has focused on developing localized and specific immunomodulatory strategies using local hydrogel-based delivery systems. In this review, we aim to emphasize the significant role of immune homeostasis in bone regeneration, explore local hydrogel-based delivery systems, discuss emerging trends in immunomodulation for enhancing bone regeneration, and address the limitations of current delivery strategies along with the challenges of clinical translation.

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1 Introduction

Bones serve multiple crucial roles in the human body, including movement, support, and protection, as well as hematopoiesis and the storage of minerals like calcium and phosphorus. Therefore, maintaining the structural and functional integrity of bones is vital for our overall quality of life. Fractures often occur as a result of significant traumatic injuries, but fortunately, bones possess a robust regenerative capacity. Unlike many other tissues, bones have the remarkable ability to heal, allowing them to regain their biological composition and nearly normal function. This extraordinary regenerative capability may be attributed to the reactivation of cellular and molecular processes observed during embryonic bone formation, which are recapitulated during the healing of fractures.[1,2]

However, despite advancements, approximately 10% of nonunion fracture cases still occur, significantly diminishing patients' quality of life and imposing a substantial financial burden on their families.[3] Therefore, conducting sustainable and in-depth studies on fracture healing mechanisms is still necessary, which can be divided into two categories. Firstly, severe trauma and excessive inflammation can result in significant bone defects that exceed the regenerative capacity of bone tissue. Secondly, disruptions in the body's immune system balance can partially impair the original regenerative ability of bone tissue. In both cases, the aforementioned excessive inflammation and immune system imbalances emphasize the crucial role of maintaining immune homeostasis during fracture healing, leading to an increased research focus on regulating immune reconstruction for bone repair. The interrelationship between the skeletal system and the immune system has long been recognized. On one hand, bone tissue serves as the main hematopoietic organ and the common origin of most immune cells, creating a similar microenvironment shared by bone cells and immune cells in the early stages. On the other

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hand, immune cell participation plays an essential role in the physiological and pathological processes of bone tissue, including cartilage formation, bone turnover, and fracture healing.[4] Consequently, a significant clinical challenge lies in efficiently, stably, and specifically regulating local immune homeostasis at the fracture site.

Bone transplantation, the traditional surgical treatment method in clinical practice, is associated with well-known drawbacks. Autologous bone grafting leads to increased morbidity at the resection site, while allogeneic bone grafting raises the risk of immune rejection and disease transmission. Moreover, these procedures are painful, expensive, and require additional surgeries. As a result, researchers began exploring biomaterials as potential bone substitutes. Initially, the focus was primarily on the low toxicity and biocompatibility of the biomaterials themselves. Biomaterials used for constructing bone tissue to repair defects can be classified into metals, ceramics, polymers, and composite materials, such as titanium alloys, silica, hydroxyapatite, and collagen, based on their chemical composition. Building upon this foundation, the concept of bone tissue engineering emerged as an attractive idea, aiming to use biomaterials to combine and control the release of active factors, hormones, drugs, and stem cells, thus inducing a favorable cellular response and enhancing the natural healing ability of the recipient tissue. This approach poses higher requirements for biomaterials, including their ability to incorporate biologically active substances, deliver drugs, carry cells, and effectively release the cargo.[5,6] Among various biomaterials, hydrogels have garnered significant attention. Hydrogels can closely mimic the natural extracellular matrix (ECM), providing an ideal environment for cell encapsulation, seeding, and homogenization. They can also serve as scaffolds for the controlled delivery of growth factors.[7] Hydrogels are polymer matrices containing cross-linked polymer chains and a substantial amount of water. They possess controllable mechanical properties and biocompatibility, making them versatile materials suitable for various applications such as immunomodulation, drug delivery, and tissue engineering. By modifying hydrogels with natural polymers or synthetic compounds, it is possible to tailor their biophysical and biochemical properties to support different cell types and optimize interactions, including cell adhesion, microstructure, and degradability.[8,9] Despite the inherent trade-off between mechanical and biological properties, with mechanical properties and biocompatibility inversely correlated with polymer concentration, hydrogels show strong potential for clinical applications. They are user-friendly, cost-effective, and readily available materials that have already found use in various areas of clinical practice, including spinal surgery and wound dressings.[10,11] It is foreseeable that in the future, more suitable hydrogels will be employed in bone tissue engineering, targeted drug delivery, and disease treatment.

In recent years, hydrogels have gained widespread attention for their ability to modulate immune microenvironments in various diseases, including cancer, cardiovascular conditions, and wound repair.[12,13] These promising findings have led to an increasing focus on hydrogel-based immunomodulation strategies for bone repair. Examples of such strategies include dual-logic hydrogels with diagnostic and therapeutic functionalities, as well as titanium hydrogels modified with ROS-responsive coatings.[14,15] However, it is worth noting that most of the existing studies are still in the experimental stage and have not yet been translated into clinical treatments. Therefore, this article aims to explore the changes in the immune microenvironment during bone repair, discuss hydrogel systems utilized for immune microenvironment modulation, and introduce relevant novel materials. The goal is to provide researchers with new perspectives for developing innovative bone repair strategies that leverage immune micro-regulation and to outline potential directions for future research in this field.

2 The prominent role of immune homeostasis in bone regeneration

Bone and the immune system have a close relationship, as several types of immune cell, including macrophages, T cells, and B cells, undergo differentiation or maturation in the bone marrow.[16] The term "bone immunology" was first introduced by Arron, J. R., and Choi, Y. in 2000 to describe the molecular mechanisms by which T cells mediate bone loss.[17] Nowadays, "bone immunology" refers to the study of the physiological and pathological interactions between the immune system and bone.[18]

The immune system plays an indispensable role in fracture healing, as multiple immune cells collaborate to regulate immune homeostasis at the site of the fracture, thereby facilitating the healing process (Fig. 1). The immune response during fractures is complex and still not fully comprehended by researchers. Since fractures can involve the contact of damaged bone tissue with the external environment, the repair process often encompasses both aseptic and bacterial inflammation. From this perspective, the immune response in fracture repair is even more intricate compared with the heart, which is one of the body's most delicate organs and typically experiences aseptic inflammation exclusively.[19] In the next sections, we will delve into the various immune cell subtypes that play pivotal roles in fracture healing (Table 1).

2.1 Macrophages and fracture repair

Tissue-resident macrophages are the primary immune cells that respond to fracture events. These macrophages, derived from monocytes and residing in the tissue, act as immune sentinels and play a crucial role in nonspecific immune regulation. For instance, microglia, the resident immune cells in the brain, surveil and protect the brain from damage, infection, and abnormal cellular activity, while also contributing to neural development and maintaining overall brain homeostasis. In the lung, alveolar macrophages diligently monitor inhaled pathogens and ensure tissue homeostasis by facilitating surfactant clearance. Also, the specific intestinal macrophages are responsible for surveying the colon for invading

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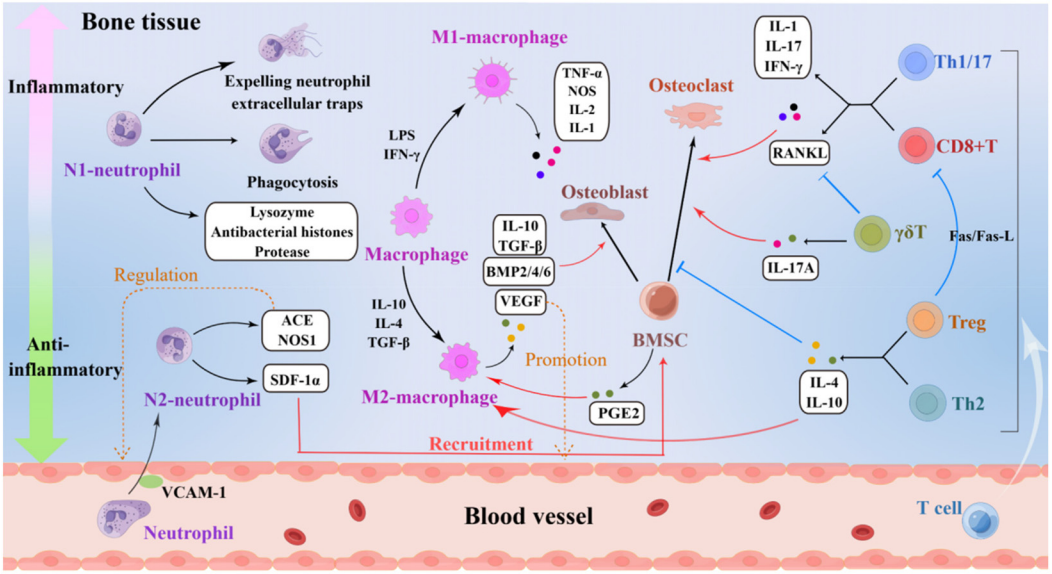

Fig. 1 The dynamic crosstalk between immune cells and tissue functional cells during the process of fracture repair. In response to a fracture, various immune cells are recruited to the site of the injury and undergo coordinated activation. This coordinated activation aims to establish a pro-repair immune microenvironment that supports osteogenic formation and promotes osteoblastic differentiation, thereby facilitating fracture regeneration.

Table 1 The classification and function of immune cells during fracture repair

Classification	Types	Functional
Macrophages	M1	(a) Promote inflammatory processes during the initial stages of fracture healing.[24] (b) Help to limit pathogen growth, invasion, and clearance of infection.[24]
	M2	Secrete anti-inflammatory cytokines to reduce inflammation during the middle and late stages of fracture healing.[25]
Neutrophils	N1	Exert pro-inflammatory effects.[41]
	N2	(a) Exert direct anti-inflammatory effects by modulating vascular factors such as ACE and NOS1.[42] (b) Recruiting BMSCs to the fracture through the SDF-1/CXCR1 axis.[43]
Mast cells	NA	Coordinating the catabolic activity, re-vascularization, and overall healing process of fractures.[49,50]
CD8+ T cells	NA	Contribute to trabecular bone loss.[52]
CD4+ T cells	Th1/17 cells	Both IFN-γ (primarily secreted by Th1 cells) and IL-17 (mainly secreted by Th17 cells) can promote osteoclastogenesis.[56]
	Treg cells	(a) Inhibit inflammation and elevate MSCs activity.[57] (b) Regulate neutrophil activity, macrophage polarization, and the cytotoxicity of other cells *via* pathways involving perforin, granzyme, or Fas-L.[58,59]
γδT cells	NA	(a) Promote bone repair through the secretion of IL-17, which stimulates the proliferation of mesenchymal progenitor cells and the differentiation of osteoblasts.[63,64] (b) Exert inhibitory effects on osteoclastogenesis by downregulating receptor activator of RANKL.[65]
B cells	NA	Influence on osteoclast maturation through regulation of the RANK/RANKL/OPG axis.[67,68]

ACE: angiotensin-converting enzyme, NOS1: nitric oxide synthase 1, BMSCs: bone marrow-derived mesenchymal stem cells, SDF-1: stromal cell-derived factor 1, CXCR1: C-X-C chemokine receptor 1, IFN-γ: interferon-gamma, IL-17: interleukin-17, Fas-L: fas ligand, RANK: receptor activator of nuclear factor kappa-B, RANKL: RANK ligand, OPG: osteoprotegerin, γδT: gamma delta T cells, NA: not applicable.

pathogens, maintaining immune tolerance to the resident microbiota, and regulating inflammation to ensure the balance of the intestinal environment.[20] Within the bone and bone marrow, various macrophage populations exist, such as osteoclasts responsible for bone resorption, erythroid island macrophages supporting erythropoiesis, and osteal macrophages located near bone-forming cells. The functional role of osteal macrophages in bone formation is currently under investigation.[20] In the normal bone environment, the majority of macrophages are quiescent tissue-resident macrophages (TRMs) that contribute to maintaining balance and stability through controlled self-renewal. When inflammation is stimulated, hematopoietic stem cells (HSCs) can be activated, leading to increased myeloid differentiation and the circulation of monocytes into bone tissue. The niche of resident macrophages can be selectively activated based on the level of inflammation, with monocyte-derived pro-inflammatory macrophages occupying available niches to preserve homeostasis. However, in cases of diabetes, an increased number of available niches are occupied by pro-inflammatory macro-

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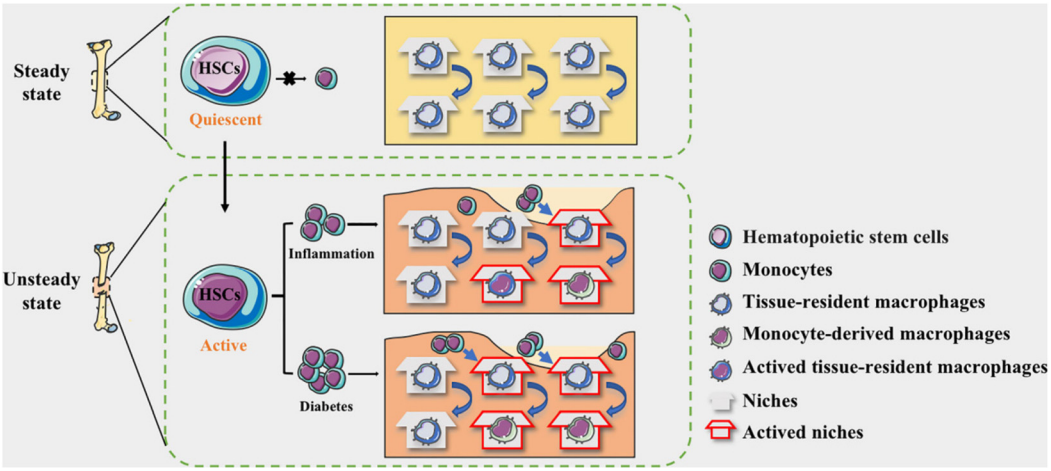

Fig. 2 Macrophages' niche in the bone tissue. Under specific conditions, macrophages occupy specific niches within bone tissues. The macrophages are mostly inactive in the physiological bone niche and maintain homeostasis through moderate self-proliferation. HSCs can activate by the inflammation stimulation and this allows monocytes to circulate into bone tissue. Based on the degree of inflammation, the niche of TRMs can activate and monocyte-derived pro-inflammatory macrophages occupy available niches to maintain homeostasis. Adapted with permission.[21] Copyright 2022, Frontiers.

phages, resulting in a prolonged pro-inflammatory state within the bone regeneration microenvironment (Fig. 2).[21] While the origins of macrophages may vary under specific conditions, their contribution throughout the entire healing process is irreplaceable. Depletion of macrophages during the early stages of a fracture may lead to reduced callus formation, which could be attributed to the involvement of macrophages in early cartilage mediation. On the other hand, depletion of macrophages during the later stages of fractures may delay bone healing, potentially due to the role of macrophages in cytokine secretion.[22,23]

The heterogeneity and polarization of macrophages have been widely recognized. In response to external stimuli, macrophages can be polarized into two functional subgroups: M1-like and M2-like macrophages. M1 macrophages are primarily activated by substances like LPS and IFN-γ. They secrete elevated levels of cytokines such as TNF-α, iNOS, IL-2, and IL-1α/β. These cytokines promote inflammatory processes during the initial stages of fracture healing, helping to limit pathogen growth, invasion, and clearance of infection. On the other hand, M2 macrophages are primarily activated by IL-10 and IL-4. They secrete high levels of anti-inflammatory cytokines such as IL-10 and TGF-β. These anti-inflammatory cytokines play a role in reducing inflammation during the middle and late stages of fracture healing, thereby facilitating tissue repair.

The regulation of inflammation *in vivo*, specifically to promote steady or subsiding inflammation, can be achieved through modulation of the M1/M2 macrophage ratio. Decreasing the M1/M2 ratio leads to the suppression of inflammation, which may be attributed to the secretion of anti-inflammatory cytokines by immunomodulatory cells.[24] Conversely, when there is a need for inflammatory progression, M2 macrophages can differentiate into osteoclasts, thereby increasing the M1/M2 ratio and promoting the existence and development of inflammation.[25] It should be noted that the classification of macrophages into M1 and M2 is not strictly polarized, and there is overlap in cytokine production. For instance, while the key anti-inflammatory factor IL-10 is predominantly secreted by M2 macrophages, small amounts can also be secreted by M1 macrophages.[26]

Under certain pathological conditions, an imbalance in the M1/M2 macrophage ratio can negatively impact bone healing. For instance, in diabetes, advanced glycation end products (AGEs) can activate the PPAR-γ/NF-κB pathway, leading to the polarization of M1 macrophages and an imbalance in the M1/M2 ratio. This can be detrimental to fracture healing. AGEs also affect the osteogenic differentiation of mesenchymal stem cells through DNA metabolism and the Wnt signaling pathway.[27] Furthermore, studies have shown that adjusting the local M1/M2 macrophage ratio can accelerate fracture healing. Researchers have explored strategies such as administration of adrenomedullary 2 (ADM2) to counter the polarization imbalance of macrophages induced by AGEs in diabetic mice, thereby enhancing bone regeneration.[28] Additionally, modulation of macrophage activity using biotic metallic ions has been investigated to coordinate the immune response in the bone tissue microenvironment and promote bone healing.[29,30]

In addition to immune function, macrophages play a role in bone formation by secreting cytokines and extracellular vesicles (EVs). For example, they secrete BMP2/4/6, which promotes osteogenesis through signaling pathways such as Smad and P38-MAPK.[31,32] Macrophages also promote the differentiation of mesenchymal stem cells into osteoblasts and enhance angiogenesis in the fracture region through the secretion of VEGF, contributing to callus remodeling.[33,34] The use of macrophage-derived EVs to promote endogenous osteogenesis has been reported, while the presence of miR144-5p in EVs from bone marrow-derived macrophages in type 2 diabetes

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has been found to hinder fracture healing by targeting Smad1.[35] Lei *et al.* has proposed two strategies for bone tissue engineering that involve regulating macrophage secretion function. These include co-culturing macrophages and bone progenitor cells *in vitro* before transplantation and designing polymers to regulate macrophage activity *in situ*. Both approaches aim to provide a continuous supply of cytokines and EVs throughout fracture healing to promote the bone repair process.[36]

2.2 Neutrophils and fracture repair

In addition to resident macrophages within the bone tissue, fractures induce an inflammatory environment that attracts a significant influx of granulocytes, particularly neutrophils. Neutrophils, the most abundant immune cells in the body, are rapidly recruited to the site of injury during pathogenic invasion and various inflammatory conditions.[37] Vascular endothelial cells at the fracture site express vascular cell adhesion molecule-1 (VCAM-1), which facilitates the recruitment of neutrophils from surrounding tissues, resulting in a high concentration of neutrophils in fracture hematomas.[38] Furthermore, neutrophils play a critical role in tissue remodeling and pathogen elimination through processes such as phagocytosis, release of extracellular neutrophilic traps (NETs), and secretion of lysozyme, antimicrobial histones, and proteases.[39] Previous studies have demonstrated that impairment of neutrophil function, as observed in a mouse fracture model with downregulated neutrophil activity, significantly hinders bone regeneration, underscoring the pivotal role of neutrophils in the fracture healing process.[40]

The concept of neutrophil heterogeneity initially emerged in the field of oncology, but recent investigations have revealed their heterogeneity in the context of inflammation. Neutrophils can be classified into two subtypes based on their impact on inflammation: pro-inflammatory neutrophils (N1) with tumor-suppressive effects and anti-inflammatory neutrophils (N2) with tumor-promoting effects.[41] This classification framework is also applicable to studies focusing on bone regeneration. N2 neutrophils exert direct anti-inflammatory effects by modulating vascular factors such as ACE and NOS1.[42] Additionally, N2 neutrophils play a crucial role in recruiting bone marrow-derived mesenchymal stem cells (BMSCs) to the fracture site. Cai *et al.* reported induced ectopic endochondral ossification using IL-8 and found a resemblance to the early stage of fracture hematoma. During ectopic endochondral ossification, neutrophils are recruited and polarized into the N2 phenotype, leading to the secretion of SDF-1 (stromal cell-derived factor-1). Through the SDF-1/CXCR1 axis and downstream signaling pathways such as PI3K/Akt and β-catenin, BMSC chemotaxis is induced.[43] Another study by the Bastian *et al.* involved the analysis of human fracture hematomas (FHs) at different time points following bone injury. Within the first 48 hours, FHs exhibited an extracellular matrix rich in fibronectin and neutrophil-derived particles, while stromal cells were only detected on the fifth day post-injury. This finding suggests that neutrophils can synthesize an "emergency extracellular matrix" during the early stages of bone injury, serving as a provisional scaffold for traditional bone repair and promoting subsequent bone regeneration.

In addition to their well-known functions, neutrophils also contribute to the recruitment of monocytes. Depletion of neutrophils in experimental settings has been shown to decrease the recruitment of inflammatory mononuclear cells. The FPR ligand LL-37 has been identified as the primary mediator of this neutrophil-mediated monocyte recruitment.[44] Activated neutrophils are capable of secreting CC-chemokines, including CCL3 and CCL4, which bind to CCR1 receptors predominantly expressed on monocytes.[45] Furthermore, neutrophils release protease 3 (PR3) that can activate adjacent cells, particularly endothelial cells, triggering the secretion of chemokines and establishing a microenvironment favorable for monocyte recruitment. PR3 is believed to upregulate intercellular adhesion molecule-1 (ICAM-1), which in turn increases the production of monocyte chemoattractant protein-1 (MCP-1) and facilitates the adhesion of neutrophils to endothelial cells.[46] Consequently, neutrophils, through the secretion of PR3, may enhance their adhesion to endothelial cells, thus promoting sustained inflammation and providing continuous chemotactic stimulation for monocytes.

2.3 Eosinophils and basophils and fracture repair

Limited research has focused on eosinophils and basophils within the context of bone regeneration, with the majority of studies emphasizing the role of tissue-resident mast cells (MCs) in fracture healing. MCs, a type of basophilic granulocyte, are primarily recognized for their involvement in mediating allergic reactions *in vivo*. Following a fracture event, MCs have been observed to accumulate, activate, undergo degranulation, and contribute to nociceptive sensitization.[47] Although the precise molecular mechanisms underlying the MCs' involvement in fracture healing remain unclear, their role is believed to be associated with inflammation.[48] Overall, MCs have a positive impact on fracture healing, as evidenced by experiments conducted on mice with MC deficiencies, such as KitW-sh and Cpa3Cre/+ mice.[49,50] However, in estrogen-deficient conditions, MCs exert a negative influence on bone repair, as MC-deficient mice have been shown to be resistant to ovariectomy-induced bone loss. This phenomenon is likely linked to MC-mediated hyper-inflammation and allergic reactions.[51]

2.4 T cell and fracture repair

T lymphocytes are responsible for mediating specific immunity, can be classified based on their surface molecules and exhibit varying effects on fracture healing.

CD8+ T cells, also known as cytotoxic T cells, possess the ability to release perforin and granzymes, and activate FAS receptors, thereby inducing apoptosis in target cells. Despite the limited research conducted on the role of CD8+ T cells in bone regeneration, conflicting results have prompted further exploration in this field. Weitzmann *et al.* reported that T cell receptor knockout experiments conducted in mice demonstrated that CD8+ T cells contribute to trabecular bone loss.[52]

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However, a contrary outcome was observed by Bernhardsson *et al.* Their experiments involved depleting the corresponding T cells through the injection of CD8 antibodies in rats, revealing an active role for CD8+ cells in cancellous bone healing.[53] These conflicting conclusions may be attributed to the use of different animal models in the experiments. Moreover, maintaining a balanced T cell population appears to be a crucial regulator of fracture healing, as an excessively elevated ratio of CD8+ effector T cells to CD4+ regulatory T cells can impair the process of fracture rebuilding (Fig. 3A).[54]

Helper T cells play diverse roles in the process of fracture repair, with particular attention given to Th1/17/Treg cells (Fig. 3B).[55] Studies have demonstrated that both IFN-γ (primarily secreted by Th1 cells) and IL-17 (mainly secreted by Th17 cells) can promote osteoclastogenesis, as evidenced by the upregulation of related mRNA and protein levels.[56] The contribution of Treg cells is indispensable for maintaining self-tolerance and preventing excessive inflammation. Through various pathways, Treg cells inhibit inflammation and promote bone regeneration, particularly by leveraging the capabilities of bone mesenchymal stem cells, thus facilitating the repair and remodeling process following a fracture.[57] Mechanistically, Treg cells exert their modulatory effects on neutrophil activity, macrophage polarization, and the cyto-

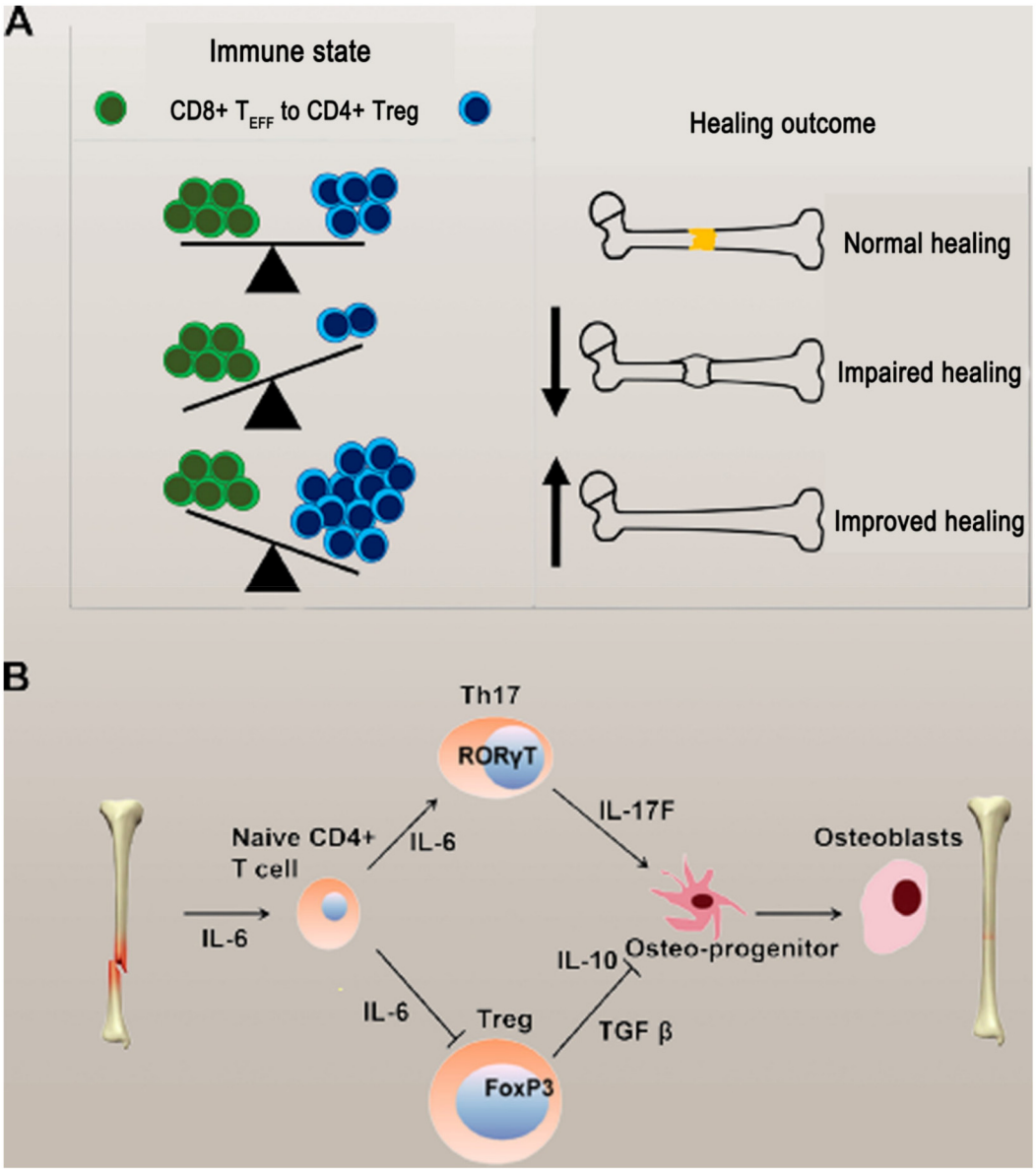

Fig. 3 The significant involvement of T cells in fracture repair. (A) The interrelationship between immune status and the outcome of healing following bone fracture. The balance between CD8+ effector T cells (CD8+ T_{EFF}) and CD4+ regulatory T cells (CD4+ Treg) influences the healing outcome. Adapted with permission.[54] Copyright 2019, Frontiers. (B) Proposed mechanistic model illustrating the role of T cells in mediating osteoblast differentiation and maturation. Adapted with permission.[55] Copyright 2012, PLOS.

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toxicity of other cells *via* pathways involving perforin, granzyme, or Fas-L.[58,59] In addition to their anti-inflammatory role in promoting bone regeneration, Treg cells can secrete cytokines such as IL-4 and TGF-β, which inhibit osteoclast differentiation.[60,61]

γδT cells, a distinct subset of T cells, exhibit innate immune properties and possess diverse biological functions including antigen presentation, cytotoxicity, and immune regulation, making them crucial in bridging innate and adaptive immunity.[62] Researchers have also focused on the role of γδT cells in the context of fracture healing. These cells can facilitate bone repair through the secretion of interleukin-17 (IL-17). Experimental evidence demonstrates the proliferation of γδT cells at the site of bone injury, accompanied by the secretion of IL-17A. Additionally, mice deficient in IL-17A exhibit impaired bone repair.[63,64] This mechanism is linked to the ability of IL-17A to stimulate the proliferation of mesenchymal progenitor cells and the differentiation of osteoblasts. Moreover, γδT cells may exert inhibitory effects on osteoclastogenesis by downregulating receptor activator of nuclear factor kappa-B ligand (RANKL), thus exerting regulatory control over bone repair.[65] While the mechanisms underlying these two pathways have been validated, further investigation is warranted to explore additional pathways through which γδT cells modulate bone repair.

2.5 B cells and fracture repair

B lymphocytes, derived from the bone marrow, are essential cells involved in specific immunity, primarily responsible for mediating humoral immune responses in the body. In the context of fracture healing, there are distinct spatiotemporal variations in the abundance of B cells within the bone tissue, indirectly suggesting their involvement in this process.[66] B cells exert their influence on osteoclast maturation through regulation of the RANK/RANKL/OPG axis, thus participating in fracture healing. Moreover, OPG, acting as a decoy receptor for RANKL, indirectly inhibits RANK signaling.[67,68] Notably, recent studies have revealed that B cells contribute to a significant portion of OPG production in the bone marrow, accounting for 64% of the total OPG production. This substantial OPG secretion by B cells enables the antagonistic effect of OPG on RANK, thereby impeding osteoclast maturation.[69] The physiological effects of OPG have been further substantiated, as its overexpression leads to osteosclerosis, while OPG deficiency results in osteoporosis symptoms.[70,71] Despite the significant attention B cells have received in the study of lymphoma-associated tumor diseases, their role in fracture healing remains relatively understudied, necessitating further exploration of the underlying mechanisms.[72]

Apart from immune cells, there exists another cell type that, although not directly engaged in immune responses, plays a vital role in regulating immune homeostasis during the process of fracture healing. These cells are known as mesenchymal stem cells (MSCs).[73] Upon attraction by chemokines such as CXCL12 and SDF-1 at the site of fracture, MSCs interact with diverse immune cells, including T cells, B cells, macrophages, and neutrophils.[74] The mechanisms underlying these interactions can be categorized into two main types. Firstly, MSCs can establish direct contact with immune cells, as observed in the interaction between MSCs and T cells. Through the binding of PD-L1 and the ligand PD-1/2, MSCs effectively inhibit T cell proliferation.[75] Secondly, MSCs can modulate cytokine secretion in response to the immune microenvironment. For example, they can induce M2-like polarization of macrophages by regulating the secretion of TGF-β.[76] When stimulated by pro-inflammatory factors like IFN-γ, TNF-α, IL-1, and IL-17, MSCs exert their anti-inflammatory function through the secretion of specific molecules. Co-culture studies involving MSCs and macrophages have demonstrated that MSCs enhance the expression of PGE2 through the iNOS and COX-2 pathways, subsequently inhibiting the production of inflammatory factors by binding to corresponding receptors on macrophages.[77] In summary, through various mechanisms, MSCs and diverse immune cells collaborate to maintain the immune balance during fracture healing, thus facilitating the orderly progression of the healing process.

3 Crosstalk between immune homeostasis and biomaterials in bone regeneration

3.1 Advantages of hydrogels in regulating immune homeostasis

When immune homeostasis is disrupted, the immune system becomes compromised, leading to prevalent pro-inflammatory conditions and potential damage or inflammation within the organism.[78] In this regard, immunotherapy has emerged as a promising therapeutic strategy for immune dysregulation-related diseases in various fields such as COVID-19, cancer oncology, organ transplantation, and trauma repair.[79,80] To effectively deliver immunotherapeutic drugs, cells, or organs, suitable carriers are required. Hydrogels have garnered significant interest as carriers due to their advantageous properties. These materials consist of a three-dimensional cross-linked network and offer biocompatibility, non-immunogenicity, and non-toxicity.[81] Compared with other drug carriers, hydrogels possess several key advantages, including low invasiveness and injectability, controlled release of encapsulated substances, biodegradability with non-toxic degradation products, and responsiveness to external stimuli. Moreover, hydrogels provide a stable immune microenvironment and reliable mechanical support for transplanted cells (Fig. 4). Ongoing research in the field of hydrogels and immunotherapy indicates that the development of highly controlled hydrogels holds great clinical promise for the future.

For implant materials that exhibit poor biocompatibility, immune recognition initiates a series of cellular processes, including sustained inflammation, foreign-body giant cell formation, fibrosis, and tissue damage in the surrounding area. These processes can have detrimental effects on the function-

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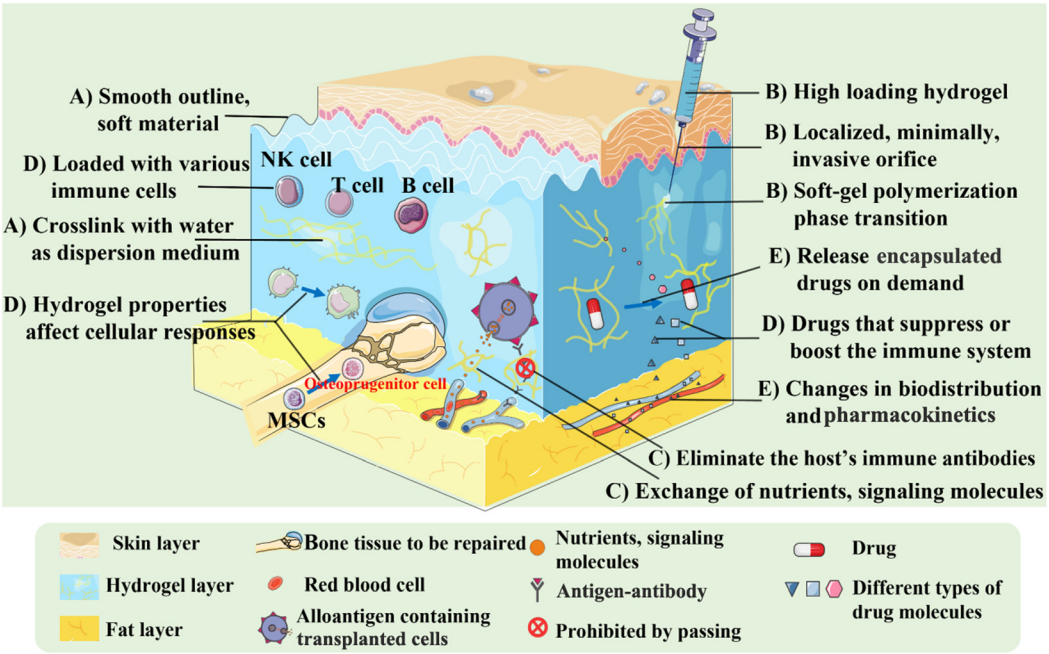

Fig. 4 The advantageous features of hydrogels in the regulation of immune homeostasis. (A) Hydrogels are polymer networks crosslinked with water as the dispersion medium, exhibiting a smooth contour and soft material properties that contribute to excellent biocompatibility. (B) Through minimally invasive surgical procedures, drug-loaded hydrogels can be introduced into the body and subsequently transformed into hydrogels using methods such as polymerization phase transition. (C) Hydrogels serve as semi-permeable barriers, enabling the exchange of nutrients and signaling molecules between host and donor cells while selectively preventing the passage of antibodies. (D) Immunotherapy commonly utilizes hydrogels as carriers for immune cells and immunomodulatory drugs. (E) Drug carriers often influence pharmacokinetics and biological distribution, and hydrogels offer the additional benefits of on-demand release and deep tissue penetration.

ality of the implant material and lead to significant pain and discomfort for the patient. Commonly used implant materials encompass hydrogels, ceramics, metals, and plastics. Hydrogels, specifically, are cross-linked polymers with water as the dispersion medium, closely resembling human tissue and thereby exhibiting remarkable biocompatibility.[82] The soft and elastic nature of hydrogels ensures a smooth and complete contour, reducing the inflammatory response and fibrosis of surrounding cells, while promoting cell viability, forming the foundation of the hydrogel's immune functionality. While hydrogels are generally considered non-toxic and non-immunogenic, caution is advised based on clinical studies that suggest the potential for delayed moderate-to-severe immune-mediated adverse effects associated with implanted hydrogels, necessitating further research for validation.[83]

Non-surgical routes of drug administration, including nasal, vaginal, rectal, and oral delivery, often present challenges due to foreign body reactions and mucosal barriers, resulting in suboptimal outcomes, drug degradation, and severe inflammatory responses.[84,85] In contrast, hydrogels offer advantages in terms of localized administration, minimal invasiveness, and high drug-loading capacity, achieved through smaller surgical wounds and syringe-based delivery. Injectable hydrogels, formed through rapid sol–gel phase transition or *in situ* chemical polymerization, enable the loading of diverse immunotherapeutic drugs, allowing direct injection into the target site for the desired effects, such as targeted modulation of the tumor microenvironment. However, conventional systemic administration typically necessitates high dosages or multiple injections, which can lead to low patient compliance and severe immune-related adverse reactions in individuals with tumors.[86]

Encapsulated cell therapy involves the transplantation of non-autologous cells, tissues, or organs in an immune-isolated manner, enabling the restoration of lost bodily functions in immune-incompatible recipients without the need for long-term immunosuppressive treatment. Hydrogels can be tailored to mimic tissue properties, facilitating the incorporation of various immune cells and drugs while selectively excluding significant immune antibodies of the host through pore size adjustment. By providing a semipermeable physiological barrier, hydrogels enable the exchange of nutrients and signaling molecules between host and donor cells, while offering mechanical support and protection against immune rejection.[87] This approach has shown promise in pancreatic transplantation and encapsulated cell therapy for nerve cells, where encapsulating the transplanted cells within semipermeable hydrogels effectively provides localized nutrition and sustains physiological functions of the pancreas or nerves, all while circumventing the challenges of immune rejection.[88]

Payloads commonly utilized in immunotherapy encompass immune cells and immunomodulatory drugs, such as

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immune checkpoint inhibitors, vaccines, immune adjuvants, adoptive cell transfer, and nonspecific immune assessment factors. These payloads play a pivotal role in intelligent drug delivery systems and immune homeostasis regulation (Fig. 5).[89,90] The response of immune cells holds significant influence over the success or failure of biomaterials following implantation. Consequently, investigating hydrogels and macrophage encapsulation systems in bone regeneration can offer a means to modulate multiple macrophage functions and enhance the performance of bone tissue engineering.[91] Diverse immunomodulatory drugs can either suppress or enhance the immune response to hydrogels. For instance, bile acids possess anti-inflammatory and immunosuppressive properties, contributing to blood pressure reduction and hypertension relief. In cancer immunotherapy, loading immunotherapy drugs can amplify the patient's immune response, leading to tumor cell elimination, inhibition of tumor metastasis and recurrence. Additionally, the payload can indirectly impact the patient's immune homeostasis by modifying the properties of the hydrogel to achieve therapeutic effects. By altering the chirality of the peptide cross-linker to impede enzymatic degradation of the scaffold, the D-amino acid cross-linked microporous annealed particle (MAP) hydrogel demonstrated the ability to activate specific immune responses, enhance the regenerative capacity of biomaterials, and induce skin regeneration.[92]

Drug carriers, including polymers, lipids, and inorganic materials, possess the ability to modify the pharmacokinetics and biodistribution of small-molecule drugs, leading to enhanced drug bioavailability and prolonged duration of action.[93] In addition to these crucial functions, hydrogels exhibit desirable features such as on-demand drug release and deep tissue penetration, which contribute to reducing inflammation and mitigating adverse immune responses, rendering them ideal for drug delivery.[94] A clinical study involving 10 008 patients with head injuries demonstrated that the administration of steroids increased mortality rates due to the systemic immunosuppressive effects throughout the body (non-brain), emphasizing the importance of local delivery limited to the injury site.[95] However, the cross-linking of the polymer network in hydrogels allows for tunability of their mechanical properties, providing the ability to protect therapeutic drugs and control their release.[96] Intelligent hydrogels responsive to endogenous or exogenous stimuli enable sustained and controlled release of immunotherapeutic drugs, minimizing unwanted side effects on normal tissues.[97] Thermal therapy facilitated by inorganic materials integrated with hydrogels can enhance cell membrane permeability through precise temperature control at the treatment site, promoting increased cellular uptake of drugs and controlled drug release within the hydrogel matrix. This approach helps to preserve the integrity of the human immune system, mitigating the risk of ablating normal cells and improving chemotherapy outcomes. Encapsulating antibiotics within hydrogels enables a selective and sustained delivery to phagocytes, enhancing cellular penetration and facilitating the treatment of intracellular infections. With their minimally invasive nature, hydrogels can be molded into desired shapes to accommodate irregular lesions and target deep tissue sites, such as those involved in cancer, wound healing, and bone repair, effectively minimizing side effects and adverse immune responses.

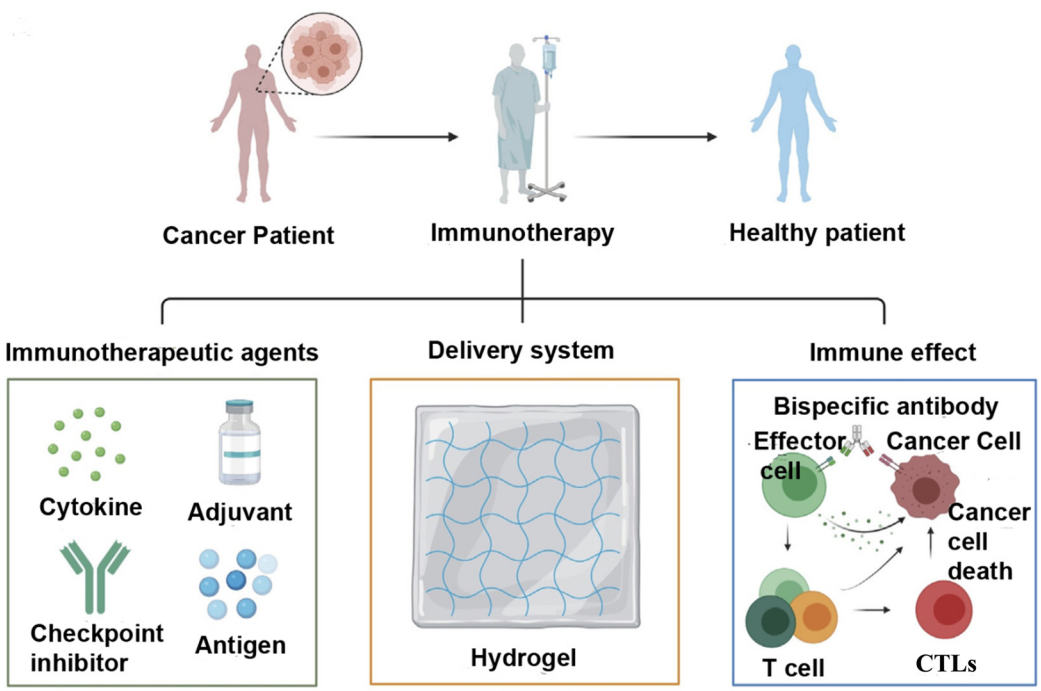

Fig. 5 Hydrogels are applied in the delivery of immunomodulatory molecules, which include cytokines, adjuvants, checkpoint inhibitors, and antigens. Adapted with permission.[89] Copyright 2021, Frontiers.

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3.2 Preparation and characteristics of hydrogels used for local delivery

Hydrogels can be strategically tailored to meet the localized drug delivery requirements for diverse therapeutic agents. A straightforward approach to achieve optimal drug loading within hydrogels involves the integration of drug delivery nanoparticles.[98,99] For instance, Kim *et al.* developed an adhesive hydrogel patch for transdermal drug delivery, where extra-large mesoporous silica nanoparticles (XL-MSNs) were employed as drug carriers.[100] Notably, the XL-MSNs played a crucial role in promoting strong adhesion between the hydrogel patch and the skin tissue. Furthermore, the multifunctionalization of drug-loaded nanoparticles within the hydrogel system has emerged as an intriguing avenue of research. For instance, a previous study designed MnO_2-coated calcium phosphate microspheres (MMS) with the ability to serve as carriers for fibroblast activating protein inhibitor (FAPi), scavengers of reactive oxygen species (ROS), and generators of oxygen. These FAPi-loaded MMS were effectively immobilized within a methacrylated PGA/methacrylated gelatin (m-PGA/GelMA) hydrogel through hydrogen bonding. Animal experiments convincingly demonstrated the remarkable capability of this multifunctional composite hydrogel to regulate the tissue microenvironment and achieve desirable therapeutic outcomes upon *in situ* implantation into osteoporotic bone defects.[101]

Another common way to increase drug delivery efficiency is introducing special microstructures into hydrogels.[102,103] The inherent hydrophilicity of hydrogels places limitations on the loading of hydrophobic drugs. To overcome this challenge, researchers have introduced hydrophobic microstructures within the hydrophilic hydrogel network.[104,105] An exemplary approach is the development of a supramolecular hydrogel system known as a host–guest macromer (HGM) hydrogel by Bian *et al.* The crosslinking of this hydrogel is established through the host–guest interaction between cyclodextrin (CD) and the aromatic groups of the gelatin polymer chain.[106,107] The molar ratio of cyclodextrin to aromatic groups in the HGM hydrogel exceeds 1, enabling the utilization of spare hydrophobic cavities within cyclodextrin as carriers for small hydrophobic drugs such as dexamethasone and icariin. Another effective strategy involves the use of amphiphilic polymers to construct hydrophobic drug-loaded hydrogels. Tong *et al.* employed atom transfer radical polymerization (ATRP) to polymerize a poly(ethylene oxide)-*block*-poly(2-(diisopropylamino) ethyl methacrylate) (PEO-*b*-PDPA) copolymer. Initially, the amphiphilic PEO-*b*-PDPA polymers self-assemble into micelle cores to encapsulate hydrophobic drugs. These micelle cores are then mixed with hydrazide-modified carboxymethyl cellulose (CMC-NH2) and oxidized carboxymethyl cellulose (CMC-CHO) to form an injectable hydrogel through a Schiff-base reaction, enabling controlled drug release.[108] Firstly, amphiphilic PEO-*b*-PDPA polymers were self-assembled to micelle cores to encapsulate hydrophobic drugs, and then these micelle cores were mixed with hydrazide-modified carboxymethyl cellulose (CMC-NH2) and oxidized carboxymethyl cellulose (CMC-CHO) to form an injectable hydrogel by a Schiff-base reaction, with controllable drug release. Additionally, electrostatic attraction can be employed for local drug delivery in hydrogels, particularly for electrically charged drugs.[109] Jia *et al.* developed a heparin-decorated hyaluronic acid-based hydrogel particle for the delivery of bone morphogenetic protein 2 (BMP-2).[110] The retention ability of the hydrogel particle for BMP-2 was strongly influenced by the concentration of heparin, as the abundant negatively charged sulfated groups of heparin polymers exerted electrostatic attraction, facilitating long-term association with BMP-2.

In contrast, drugs, particularly peptide drugs, can be directly grafted onto the backbone of hydrogel networks.[111] Zhang *et al.* employed methacrylated icariin (Ica-MA), methacrylated hyaluronic acid (HA-MA), and type I collagen (Col I) to fabricate an icariin-conjugated hyaluronic acid/collagen hydrogel for reconstructing the osteochondral interface.[112] During the gelation process, icariin was incorporated into the network of the hydrogel through radical polymerization involving the methacrylate groups of Ica-MA and HA-MA. The grafted icariin was found to promote both chondrogenesis and osteogenesis, facilitating the repair of the osteochondral interface. Similarly, Yu *et al.* grafted a novel calcitonin gene-related peptide (CGRP) onto a photocrosslinking hyaluronic acid-based hydrogel *via* dynamic covalent hydrazone bonds.[113] This enabled the hydrogel to enhance the proliferation and osteogenesis of BMSCs through sustained release of bioactive CGRP peptides. Moreover, besides their therapeutic effects, these grafted drugs also played a crucial role in the crosslinking of the hydrogel. Cai *et al.* reported an amphoteric copolymer-based hydrogel for bone regeneration.[114] The hydrogel was synthesized *via* one-step radical polymerization involving (*N*-acyl glycinamide) (NAGA), anionic acrylate alendronate (AcAln), and cationic 2-(acryloyloxy)-*N*,*N*,*N*-trimethylethan-1-aminium (DMAEA-Q). In addition to promoting BMSCs osteogenesis, AcAln contributed abundant bisphosphate groups that formed hydrogen bonds between dual bisphosphate groups, and ionic pairs between bisphosphate groups and the quaternary ammonium groups of DMAEA-Q. These interactions served as the main crosslinking mechanisms, ensuring the mechanical flexibility of the hydrogel.

In addition to enhancing drug loading efficiency, researchers also emphasize the design of controllable drug release strategies.[115] Responsive drug release from hydrogels can be achieved through two primary modes. The first mode involves utilizing external stimuli, such as light, heat, and magnetism, to trigger drug release from hydrogels.[116] Among these external stimuli, light is the commonest used one because of its easy accessibility and convenient operability.[117] Ossipov *et al.* developed a photosensitive prodrug-grafted hyaluronic acid hydrogel using orthogonal chemistry.[117] By employing a photolabile *ortho*-nitrobenzyl linker, dopamine was grafted as a model drug to the hydrogel backbone. Under UV light exposure, the photosensitive linker rapidly broke, enabling controlled release of the model drug. The second mode is based on the bio-responsiveness of hydrogels to internal phys-

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iological signals such as pH, redox potential, enzymes, and glucose.[118,119] Wu *et al.* fabricated a dual-sensitive hydrogel composed of chitosan, hyaluronic acid, and β-sodium glycerophosphate.[120] Gelation of this system was triggered by body temperature sensitivity conferred by β-sodium glycerophosphate, while the acid-responsive drug release was controlled by the protonation of chitosan. Glucose-sensitive hydrogels, on the other hand, are often employed to achieve responsive insulin release for diabetes patients. Gu *et al.* utilized a core–shell microneedle hydrogel for transdermal self-regulated insulin delivery.[121] As the glucose concentration increased, the encapsulated glucose oxidase catalyzed its conversion into H_2O_2. Subsequently, H_2O_2 triggered the cleavage of the linker *N*1-(4-boronobenzyl)-*N*3-(4-boronophenyl)-*N*1,*N*1,*N*3,*N*3-tetramethylpropane-1,3-diaminium (TSPBA) between the insulin and poly(vinyl alcohol) (PVA), resulting in the release of insulin into the bloodstream as required.

4 Local delivery hydrogels to restore immune homeostasis

4.1 Delivery of small molecules

The use of hydrogels for the delivery of interleukin-4 (IL-4), a cytokine known for its anti-inflammatory properties, has been extensively studied to promote bone regeneration.[122] Researchers have reported that local delivery of IL-4 using calcium-enriched gellan gum hydrogel facilitated bone defect regeneration in rats by modulating macrophage polarization, promoting osteogenic differentiation of bone MSCs, and reducing cell apoptosis.[123] Another study by Zou *et al.* involved loading IL-4 and bone morphogenetic protein-2 (BMP-2) onto graphene oxide (GO) and embedding them into a carboxymethyl chitosan (CMC)/poly(ethylene glycol) diacrylate (PEG-DA) hydrogel. This hybrid hydrogel controlled the release of IL-4, thereby transforming M1 macrophages into M2 macrophages.[124] In a separate investigation, Krieger *et al.* demonstrated that delivery of stromal-derived factor-1α (SDF-1α) using PEG-DA hydrogel stimulated chemotaxis of anti-inflammatory monocytes and supported the growth of microvascular networks.[125] Lipid mediators, known to regulate inflammation and bone regeneration, have also been utilized.[126] It has been demonstrated that collagen/Pluronic F127 hydrogel loaded with resolvin D1 (RvD1), derived from omega-3 docosahexaenoic acid, effectively reduced inflammation and promoted calvarial defect repair in rats.[127] Hydrogels have also been employed as delivery carriers for sustained release of anti-inflammatory drugs in bone tissue regeneration. For instance, Chauhan *et al.* loaded dexamethasone (Dex) into an oxidized pullulan (OP)/PEG hydrazine hydrogel, providing pH-sensitive sustained release and improved chronic inflammatory conditions.[128] Moreover, several traditional Chinese medicines have shown promise in immune response regulation and bone healing. Guo *et al.* demonstrated that delivery of baicalin with a GO-demineralized bone matrix (DBM) hybrid scaffold induced macrophage differentiation into the M2 type, promoting angiogenesis, osteogenesis, and calvarial bone regeneration *in vivo*.[129] Puerarin has been reported to enhance bone formation through inhibition of osteoclast activities and stimulation of osteoblast differentiation.[130,131] Recent evidence suggests that delivery of puerarin *via* GelMA hydrogel reconstructed the pelvic floor, associated with reduced expression of inflammatory cytokines (interleukin-3 (IL-3) and interleukin-6 (IL-6)) and matrix metalloproteinase-2/9 (MMP-2/9), thereby minimizing multiple inflammations.[132] However, further research is needed to directly investigate the immunomodulatory effects of puerarin delivery during bone regeneration.

4.2 Delivery of biologics

Biologics, which are produced using biological engineering technology and mimic the structures of antibodies, receptors, and cytokines, have gained significant attention for their ability to modulate signal transduction pathways.[133] In the field of skeletal diseases, the utilization of biologics has increased due to their high specificity towards molecular targets.[134] In the context of bone regeneration, the negative impact of tumor necrosis factor (TNF)-α overexpression led Timmen *et al.* to investigate the use of an anti-TNF antibody (infliximab) for fracture treatment, revealing significant improvements in fracture healing upon TNF-α blockade.[135] Similarly, Dixit *et al.* examined the effects of an anti-IL-17 antibody on bone tissue regeneration, observing enhanced expression of osteogenic markers and reduced oxidative stress at the defect site.[136] Although these studies demonstrated the potential of biologics to promote bone regeneration, their administration without suitable carriers may not ensure sustained effects. To address this, hydrogels have emerged as effective delivery carriers for enhancing the therapeutic outcomes of biologics. Chen *et al.* implanted an injectable, self-healing, adhesive HA@SDF-1α/M2D-Exos hydrogel, which synchronously and sustainably releases stromal cell-derived factor-1α (SDF-1α) and M2 macrophage-derived exosomes (M2D-Exos), thereby promoting osteogenesis and angiogenesis, offering a promising modality for accelerating bone repair by addressing the challenges of nonunion fracture (Fig. 6).[137] In addition to physical entrapment, chemical conjugation serves as another effective strategy for drug encapsulation and release in hydrogel-based delivery systems for biologics. Notably, recombinant protein cross-linked hydrogels have been explored for bone regeneration. For instance, the incorporation of recombinant collagen peptide microspheres into alginate hydrogels enabled *in situ* gelation and delivery of BMP-2, inducing bone formation.[138] However, the application of hydrogels for delivering recombinant proteins with immunomodulatory activity in bone repair remains limited, warranting further research in this area.

The delivery of biologics using hydrogels shows significant promise due to the highly compatible nature of hydrogels with nucleic acids and proteins, which are susceptible to environmental conditions. However, achieving effective and controlled release of biologics remains a challenge. A key area of research

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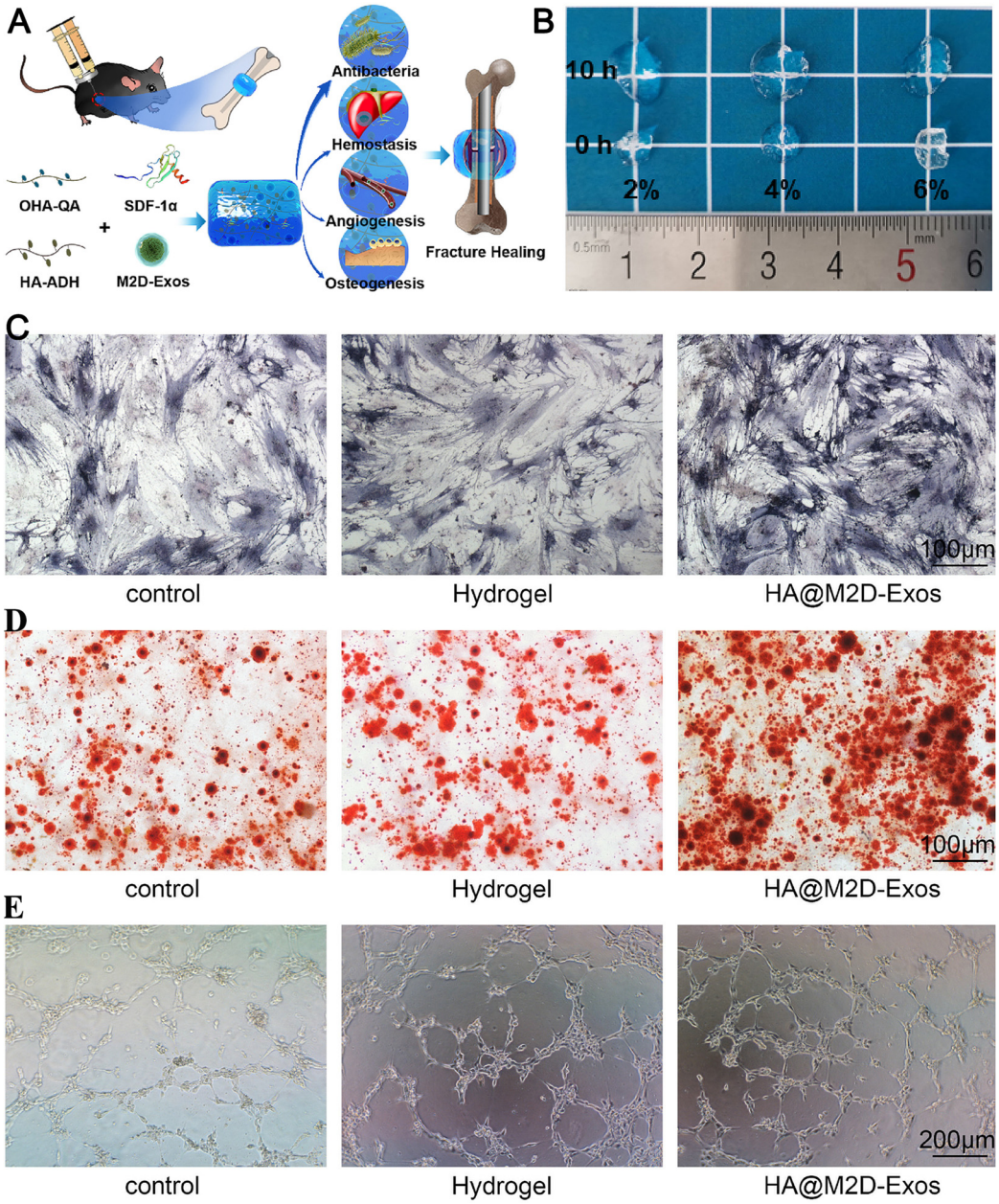

Fig. 6 The immunomodulatory effects of biologics loaded in hydrogels for bone regeneration. (A) Schematic image illustrating the effect of HA@SDF-1α/M2D-Exos hydrogels on fracture repair. (B) Representative image of the swelling equilibrium of an HA-based hydrogel. (C and D) ALP staining and Alizarin Red staining of treated HMSCs after osteogenic induction following different treatments (scale bar = 100 μm). (E) Tube formation assay of HUVECs in different groups. Adapted with permission.[137] Copyright 2023, Elsevier B.V.

involves the development of stimuli-responsive hydrogels capable of responding to external cues such as pH, reactive oxygen species (ROS), heat, and light, enabling targeted delivery of biologics in a spatially and temporally controlled manner.[139] While there are still challenges to address, the investigation of hydrogel systems for delivering biologics with immunoregulatory functions holds great potential for modulating local immune responses and promoting enhanced bone regeneration.

4.3 Delivery of small EVs

EVs are small membrane-encapsulated particles released by cells that serve as intercellular messengers, facilitating the transfer of proteins and genetic information. Their excellent biosafety and stability have recently garnered significant attention in the fields of bone regeneration and immune modulation.[140] However, achieving a long-lasting retention and controlled release at defect sites using appropriate carriers

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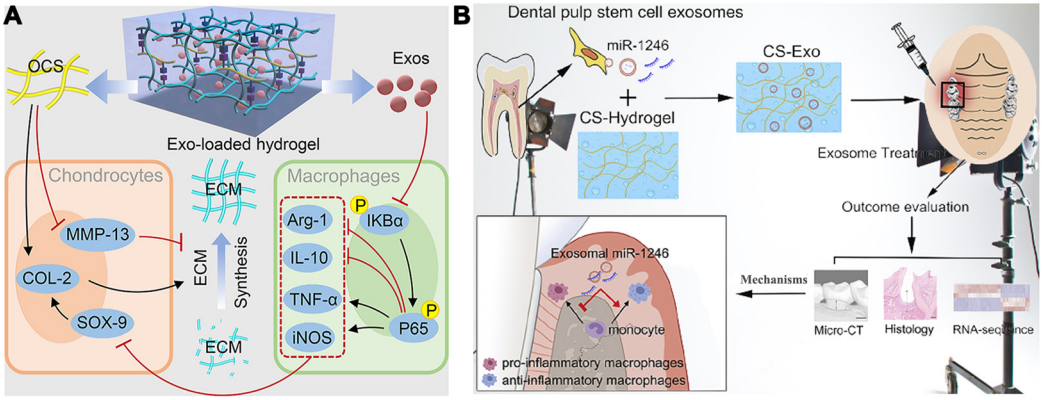

Fig. 7 The immunomodulatory effects of EVs loaded into hydrogels for bone regeneration. (A) The diagram depicts the utilization of hydrogel loaded with exosomes derived from BMSCs to facilitate repair of growth plate injuries by suppressing inflammation. Adapted with permission.[143] Copyright 2022, Elsevier. (B) The miR-1246 present in DPSC exosomes stimulates the polarization of M2 macrophages.[144] Copyright 2020, Elsevier B.V.

remains a challenge. The utilization of hydrogels as delivery vehicles for EVs offers a novel approach to modulating the local immune response and enhancing bone regeneration.

Various sources of EVs have been explored for the treatment of bone defects, with a particular focus on the immunomodulatory properties of mesenchymal stem cell (MSC)-derived EVs.[141] Li *et al.* investigated the therapeutic potential of adipose-derived MSC EVs (ADSC-EVs) loaded with gelatin nanoparticles for traumatic bone defects. They demonstrated that ADSC-EV delivery inhibited M1 macrophage polarization while promoting M2 macrophage polarization, resulting in enhanced bone healing.[142] Similarly, Guan *et al.* utilized EVs derived from BMSCs loaded in extracellular matrix (ECM)-mimic hydrogels to facilitate growth plate injury repair in rats. The EVs inhibited inflammation and stimulated ECM synthesis, leading to successful repair of the injury (Fig. 7A).[143] In another study, Shen *et al.* incorporated EVs derived from dental pulp stem cells (DPSCs) into chitosan hydrogels. The presence of miR-1246 in DPSC exosomes effectively induced M2 macrophage polarization, reduced inflammatory cytokines, and accelerated alveolar bone healing and periodontal epithelium regeneration in mice (Fig. 7B).[144] Additionally, EVs derived from macrophages have shown promise in modulating the immune microenvironment and bone regeneration. It has been observed that EVs from M0 and M2 macrophages promote bone repair, while EVs from M1 macrophages inhibit bone regeneration.[35] It has been demonstrated that M0 and M2 macrophage-derived EVs promote bone repair and that M1 macrophage-derived EVs inhibit bone regeneration.[145] Therefore, delivering EVs derived from M0 and M2 macrophages could serve as a potential strategy to regulate the immune microenvironment and enhance bone regeneration.

4.4 Strategies for cell-based immunomodulation

MSC-based therapy has emerged as a promising strategy for bone regeneration due to the inherent ability of MSCs to maintain tissue homeostasis.[146] The regenerative effects of MSCs in bone tissue engineering can be achieved through both endogenous MSC recruitment and exogenous MSC transplantation.[147] Recent evidence indicates that MSCs exert immunomodulatory actions, in addition to their role in differentiation and repopulation, further enhancing bone regeneration.[148] Apart from BMSCs, MSCs derived from adipose tissue (ADSCs), dental pulp (DPSCs), and umbilical cord Wharton's jelly (WJMSCs) have also been explored for the treatment of bone defects. MSCs possess low immunogenicity and can dampen immune responses. Their immunomodulatory actions involve interactions with various immune cells, including macrophages, lymphocytes, and natural killer (NK) cells, facilitated by the secretion of cytokines such as 2,3-dioxygenase (IDO), IL-6, interleukin-10 (IL-10), and nitric oxide synthase (iNOS) within the inflamed tissue microenvironment.[149,150] Hydrogels, with their unique physiochemical properties and three-dimensional structure, offer inherent advantages in mimicking the natural extracellular matrix (ECM) and supporting MSC adhesion and survival on a large scale.[151] Consequently, the delivery of MSCs using hydrogel culture systems represents an effective approach for modulating the immune response and enhancing bone regeneration.

For instance, Seebach *et al.* seeded BMSCs on fibrin hydrogels and implanted these composite constructs into femoral bone defects. Compared with cell-free fibrin hydrogels, the BMSC-seeded fibrin implants exhibited enhanced macrophage and endothelial progenitor cell migration into the hydrogel, leading to accelerated tissue maturation and blood vessel formation during the early stages of healing.[152] Ji *et al.* developed a hybrid scaffold by incorporating BMSC-encapsulated thermosensitive hydroxypropyl chitin hydrogel (HPCH) into a 3D-printed poly(ε-caprolactone) (PCL)/nanohydroxyapatite (nHA) scaffold for the treatment of calvarial defects in rats. This hybrid scaffold effectively promoted BMSC osteogenic differentiation and polarization of macrophages towards the M2 phenotype, thereby facilitating osteoinduction of BMSCs and angiogenesis (Fig. 8).[153] To enhance the immunomodulatory

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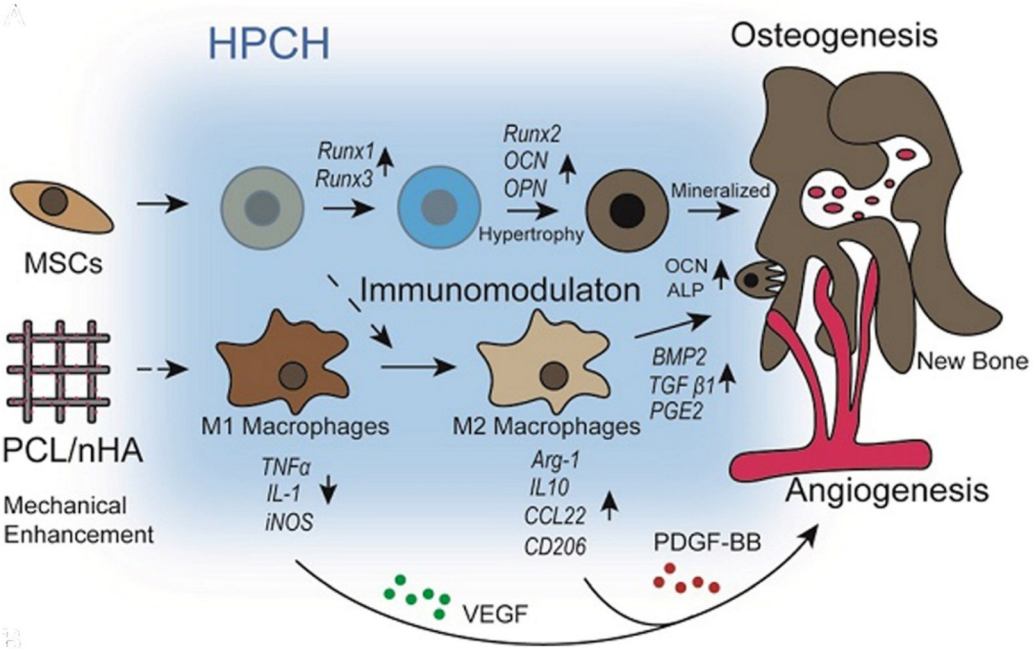

Fig. 8 The diagram depicts the BMSC-encapsulated HPCH/PCL/nHA scaffold designed for bone regeneration. Adapted with permission.[153] Copyright 2020, Ivyspring.

effects of MSCs, Ueno *et al.* engineered lentivirus-transduced BMSCs overexpressing IL-4 and delivered these IL-4-modified BMSCs within a gelatin-based microribbon hydrogel to critical-size long bone defects in mice. The IL-4 BMSCs continuously released IL-4 from the microribbon hydrogel, attracting macrophages to the hydrogel and polarizing them towards the M2 phenotype, while not inhibiting M1 macrophage polarization. This localized immune modulation resulted in enhanced bone formation and regeneration after a 6-week period.[154]

The effectiveness of MSC-based bone regeneration is not always assured due to the loss of cellular properties, including immunomodulatory activity, during *in vitro* expansion and the unpredictable behavior of MSCs in diseased microenvironments. To enhance MSC-based bone regeneration, the development of biocompatible hydrogels capable of preserving MSC properties is a promising strategy. Furthermore, it has been discovered that the stiffness of hydrogels can influence the immunomodulatory efficacy of MSCs. MSCs cultured on soft matrices demonstrate superior anti-inflammatory performance compared with those on stiff matrices.[155] Therefore, the mechanical response of MSCs to hydrogels must be considered when designing MSC-embedded hydrogels for immune regulation. Additionally, MSCs are a heterogeneous population, and not all MSCs possess the same immunomodulatory abilities.[156] Thus, the selection of appropriate cell types is crucial for the success of MSC-based bone regeneration. Recent evidence indicates that Wharton's jelly-derived MSCs (WJMSCs) exhibit enhanced immunomodulatory activity compared with BMSCs, highlighting the potential of delivering WJMSCs with hydrogels to promote bone regeneration.[157]

5 Combination of immunomodulatory regeneration with other techniques

Combination therapy presents a potential avenue to enhance treatment efficacy and reduce the duration of treatment. In the context of bone regeneration, hydrogel-based immunotherapy offers the potential to improve therapeutic outcomes by modulating macrophage functions to regulate immune responses and prevent bone resorption. However, the integration of immunotherapies with other techniques within novel multifunctional hydrogels holds even greater promise for achieving efficient bone repair through synergistic effects. One of the major challenges in bone tissue engineering is the occurrence of bone infections, which contribute to high morbidity rates and significant healthcare burdens.[158] Bacterial infections disrupt the balance of bone-forming osteoblasts and bone-resorbing osteoclasts due to the accumulation of inflammatory mediators, greatly impacting the process of bone regeneration.[159] The combination of antibacterial and osteoimmunomodulatory activities represents an innovative strategy that can simultaneously promote osteogenesis and eliminate bacterial presence in defect areas. Ou *et al.* developed a hybrid hydrogel composed of gelatin methacrylate (GelMA), which closely mimics the natural extracellular matrix (ECM) environment. This hydrogel was loaded with nanosilver (nAg), an effective broad-spectrum antibacterial agent, and halloysite nanotubes (HNTs) to facilitate bone regeneration. The nAg/HNTs/GelMA hydrogel demonstrated notable osteoimmunomodulatory and antibacterial properties (Fig. 9). It exhibited significant inhibition zones against *Escherichia coli* (*E. coli*) and

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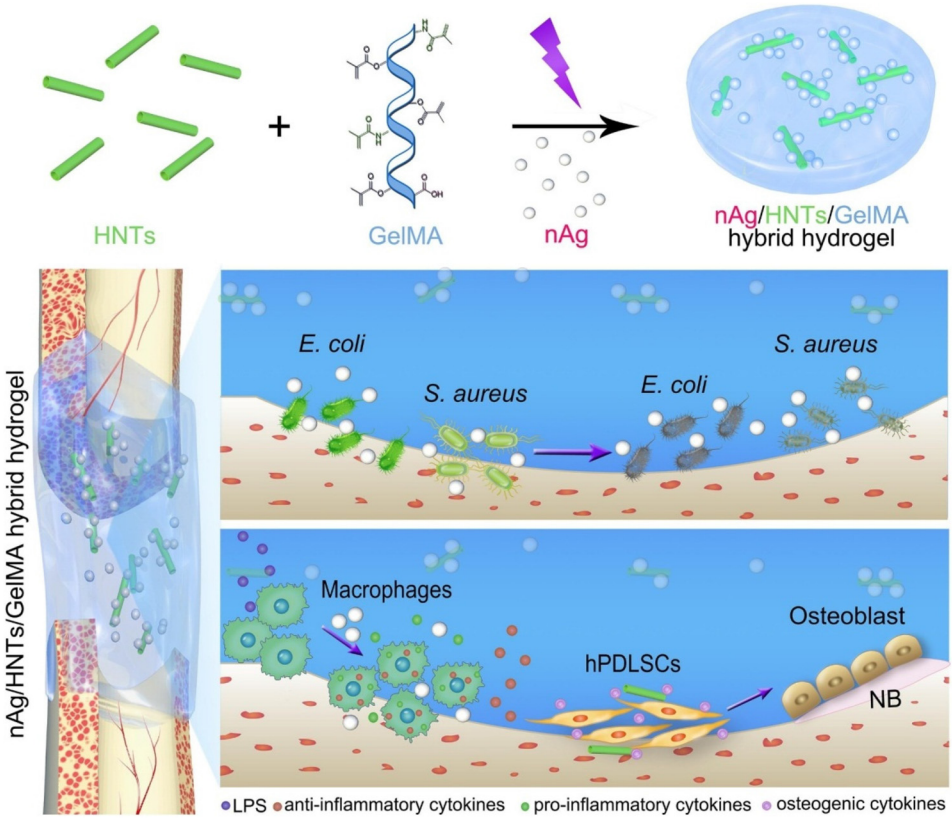

Fig. 9 Schematic illustration of the nAg/HNTs/GelMA hydrogel, including its fabrication procedure, osteoimmunomodulatory properties, and antibacterial activity (LPS: lipopolysaccharide; hPDLSCs: human periodontal ligament stem cells; NB: new bone). Adapted with permission.[160] Copyright 2020, Elsevier B.V.

Staphylococcus aureus (*S. aureus*). Moreover, in infected bone defects, the nAg/HNTs/GelMA-treated group showed substantially fewer *S. aureus* colonies compared with other groups, along with inhibited inflammatory reactions *in vivo*. The incorporation of nAg in the hydrogel resulted in reduced expression of IL-1β and IL-6, while increasing IL-10 expression compared with other groups. This enhanced osteoimmunomodulatory stability under infectious conditions and mitigated the inflammatory responses. Furthermore, the nAg/HNTs/GelMA group exhibited greater osteogenesis as evaluated by micro-computed tomography (micro-CT), bone mineral density, and bone volume fraction (BV/TV) at the two-month mark. Taking these together, the reported platform demonstrates synergistic antibacterial and osteoimmunomodulatory effects for bone regeneration, particularly in the reconstruction of infected bone defects.[160] Another study explored the antibacterial, immunomodulatory, and osteogenic properties of a composite hydrogel consisting of polylactic-glycolic acid (PLGA) microspheres embedded within a porcine small intestinal submucosa (SIS) hydrogel. This platform was designed with sequential drug release capabilities, incorporating LL-37 and W9 peptides. The presence of LL-37 peptide provided multifunctionality, including antibacterial activity, cell recruitment, and immunoregulation, while the W9 peptide promoted calcium deposition and upregulated osteogenic gene expression. Consequently, this prepared platform exhibited great potential for accelerated bone regeneration through immune regulation and antibacterial effects.[161]

In addition to bone infection, the formation of orthopedic biofilms and weak post-surgical osseointegration pose significant challenges in orthopedics, potentially leading to implant failure.[162] Addressing both biofilm infections and osseointegration simultaneously has therefore become crucial. To this purpose, Li *et al.* developed an adhering hydrogel consisting of poly(vinyl alcohol) (PVA) modified with chitosan (Cs) and polydopamine (PDA), referred to as PCP, along with the nitric oxide (NO) donor *S*-nitrosuccinic acid (RSNO). This hydrogel was then coated on a red phosphorous (RP) nanofilm deposited on a titanium implant (Ti-RP/PCP/RSNO), synergistically combining photothermal therapy (PTT) and immunotherapy to eliminate infection and enhance osteogenesis.[163] PTT is a novel local therapy method that utilizes light-to-heat conversion, resulting in the non-invasive and low-toxicity destruction of bacterial cells.[164,165] The fabricated hydrogel exhibited significant heat generation (approximately 53.1 °C) under near-infrared (NIR) irradiation, owing to the photothermal activities of RP and PDA. Subsequently, the Ti-RP/PCP/RSNO-treated group achieved over 93.1% removal of an MRSA biofilm mass after NIR irradiation, followed by a 2-day biofilm incubation period. Furthermore, the released NO at a low concentration

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exhibited antibacterial effects, induced M1 polarization of macrophages, upregulated the expression of osteopontin (Opn) and osteocalcin (Ocn) genes involved in osteoblast differentiation, as well as TNF-α expression, thereby enhancing osteogenic capability. Consequently, the prepared hydrogel was proposed as a novel biomaterial for simultaneous biofilm elimination and promotion of osteogenesis through phototherapy and immunotherapy.[163] Additionally, PTT's photothermal effect can generate acoustic waves, facilitating photoacoustic imaging for visualizing targeted sites in bone defects.[166] Regarding this property, Jiang *et al.* developed an injectable hydrogel based on an alginate/sericin/graphene (Alg/Ser/GO) framework with HRP/H_2O_2 enzymatic cross-linking. Silk sericin promoted macrophage infiltration, M2 polarization, and the transition from the M1 to M2 phenotype, which is associated with anti-inflammatory macrophages that aid angiogenesis and bone regeneration. GO, the oxidized form of graphene, acted as a photothermal agent while also improving osteogenic differentiation and bone regeneration. Monitoring the changes in temperature under NIR irradiation confirmed the higher temperature of the injected hydrogel. The mild heat generated by the photothermal effect under NIR irradiation enhanced the differentiation of BMSCs and facilitated bioimaging for tracking the location of the injected hydrogel. Moreover, GO exhibited great potential for photoacoustic-based imaging to monitor the hydrogel. Ultimately, the fabricated injectable hydrogel demonstrated a synergistic effect on bone healing after 8 weeks, offering traceability and biocompatibility. By combining imaging modalities with therapeutic agents, enhanced strategies for bone regeneration treatment are realized.[116]

6 The limitations of current hydrogel delivery strategies and challenges of clinical translational application

6.1 The less-than-desirable mechanical strength of hydrogels

Despite their advantages as a delivery system, hydrogels still face certain limitations that require further attention. One major concern is the inadequate mechanical strength of natural polymers such as chitosan, fibrin, and collagen, despite their excellent biocompatibility. Alginate hydrogels, for instance, are commonly used in bone tissue engineering due to their unique properties such as low toxicity, affordability, and widespread availability. However, meeting the mechanical strength requirements for bone tissue engineering, which typically range from 25 to 70 MPa for compressive strength and around 200 ± 36 MPa for cortical bone, poses a challenge.[167] To address this issue, chemical crosslinking networks have been extensively explored to enhance the mechanical properties of hydrogels, resulting in improved strength and durability.[168] Synthetic polymers, although they offer certain advantages by circumventing some limitations, such as unpredictable immune responses and poor degradation, still require solutions to overcome these challenges.[169] Thus, finding the appropriate polymer composition and crosslinking methods to enhance the properties of hydrogels for bone tissue engineering remains a long-term goal.

6.2 The unstable release efficiency of hydrogels

Achieving a sustained and stable release of therapeutic agents from hydrogels is crucial for promoting the spontaneous bone regeneration process. However, the breakdown of hydrogels by biological factors can hinder this process. To address these limitations, one approach is to enhance the binding affinity of hydrogels through physical and chemical cross-linking methods. In physically cross-linked hydrogels, the release rate of agents is primarily influenced by factors such as viscosity, degradation, and weak interactions. On the other hand, chemical crosslinking involves the formation of covalent modifications between hydrogels and agents. However, it is important to consider that covalent binding might compromise the activity of the agents. To overcome this drawback, stimuli-responsive hydrogels that can undergo changes in response to the local environment offer a potential solution. These hydrogels can exhibit desired modifications without impairing the activity of the agents.[170] Some studies have demonstrated the feasibility of independently and simultaneously releasing multiple bioactive proteins from hydrogels through independent affinity interactions.[171] In conclusion, achieving precise control over agent release poses a significant challenge in the development of ideal hydrogels.

6.3 The unsetting influence of hydrogels on cells

The application of hydrogels loaded with stem cells for bone regeneration has been reported as promising. However, several challenges must be addressed. Firstly, hydrogels face difficulties in accurately replicating the complex microenvironment of the fracture site, leading to potential deviations in the growth of stem cells. Secondly, the structure of hydrogels may cause undesired differentiation of stem cells into specific cell lineages. Additionally, achieving a precise and targeted delivery of cargo to specific cells within the hydrogel is challenging, potentially impacting the behavior of other cells and introducing unpredictable side effects. Therefore, it is crucial to thoroughly assess the physical and chemical properties of hydrogels, including their morphology, stiffness, and biodegradability, to ensure their suitability for bone regeneration.[172]

6.4 The clinical research of hydrogels

In recent years, a wide range of hydrogels have been developed for both *in vivo* and *in vitro* studies, demonstrating promising results that warrant further investigation in clinical studies. Additionally, researchers have made advancements in modifying hydrogels to meet clinical demands. For instance, microfluidic technology enables the fabrication of micrometer-sized hydrogels with diverse geometries and morphologies, expanding their biomedical applications.[173] Despite these advancements, challenges persist in large-scale manufacturing of hydrogels, particularly in maintaining their stability and

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repeatability at an industrial level. Sterilizing hydrogels presents another challenge due to their hydration properties. Comprehensive sterilization protocols are necessary to ensure that source materials and production processes are free from contamination. Terminal sterilization methods, both physical and chemical, can be employed as long as they do not compromise the performance and therapeutic efficacy of the hydrogels. Careful selection of the appropriate sterilization method and thorough testing of hydrogel activity are imperative.[174,175] Another challenge in clinical research on hydrogels is storage. Hydrogels are typically stored in hydrated or dehydrated states based on their properties.[176] However, bioactive components within hydrogels may undergo alteration when exposed to environmental temperatures or during long-term storage. Therefore, it is essential to assess the activity of the bioactive components before utilizing the hydrogels.

The utilization of hydrogels necessitates adherence to a series of tests outlined by the International Organization for Standardization (ISO), encompassing assessments of the material's chemical properties, cytotoxicity, and pre-clinical trials.[142] These extensive pre-clinical trials and comprehensive clinical evaluations are essential, albeit time-consuming and costly, particularly when hydrogels are loaded with bioactive components.[102] Consequently, the financial burden associated with the translational process of hydrogels from laboratory to clinical applications can be substantial.

7 Concluding remarks and future challenges

As researchers continue to advance in their understanding of hydrogels and foster increased collaboration, there has been a remarkable expansion in the application of hydrogels for various diseases. This progress, coupled with the rapid translation of hydrogels into human clinical trials, has ignited enthusiasm within the scientific community.[177] However, there are several challenges that need to be addressed for the successful clinical translation of hydrogels and to shape their future perspectives. One major challenge is the limited understanding of the immune environment and the optimal design of hydrogels to achieve desired therapeutic outcomes. Although hydrogels hold promise as immunomodulatory platforms for tissue regeneration, further research is required to unravel the underlying mechanisms of immune response and enhance the effectiveness of hydrogel-based therapies. Drawing inspiration from the extensive research on tumor microenvironments, innovative technologies and perspectives can provide valuable insights for investigating the immune microenvironment during fracture repair. Key tools such as single-cell sequencing and genetic mouse lines will play a crucial role in advancing this field of research. Another crucial aspect to consider is the role of cell-to-cell communication in tissue repair. The viability of stem cells significantly impacts the activation of immune cells, highlighting the importance of designing hydrogels that facilitate effective cell communication. Additionally, the interplay between the immune microenvironment of bone and the overall systemic microenvironment remains poorly understood. It is essential to investigate microenvironmental changes during bone repair in diverse populations, including individuals with specific health conditions, such as osteoporosis, obesity, diabetes, or vitamin deficiencies.

While unraveling the immune microenvironment during bone repair presents significant challenges, it also opens up opportunities for the development of more effective and personalized hydrogels for immunotherapy. Future research should focus on addressing these challenges to advance the clinical translation of hydrogels and maximize their therapeutic potential. By gaining a deeper understanding of immune mechanisms and optimizing hydrogel design, researchers can pave the way for improved treatments and combining personalized approaches to tissue regeneration.

In summary, while the application of hydrogels in immunomodulatory regeneration of bone defects shows promise, addressing the challenges associated with their clinical translation is essential. Hopefully, by bridging the knowledge gaps and embracing innovative technologies, researchers can unlock the full potential of hydrogels in clinical settings, revolutionizing the field of regenerative medicine.

Conflicts of interest

The authors declare no competing interests.

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