REVIEW

Immunomodulatory Effects and Mechanisms of Two-Dimensional Black Phosphorus on Macrophage Polarization and Bone Regeneration

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Abstract: The repair of bone defects poses a significant challenge within the realm of clinical medicine. However, with the advent of various emerging biotechnologies, bone tissue engineering (BTE) has emerged as a promising discipline that offers innovative therapeutic strategies to address bone defects. Among the novel biomaterials being explored, two-dimensional (2D) black phosphorus (BP) has attracted considerable attention due to its advantageous properties, which include antimicrobial activity, drug delivery capabilities, and effective photothermal conversion. These properties render BP an excellent candidate for BTE applications. Recent studies have indicated that BP possesses remarkable immunomodulatory properties that influence bone regeneration, profoundly impacting the transformation of the osteoimmune microenvironment, thereby guiding the process of bone remodeling. Macrophage is a principal component of the osteoimmune microenvironment, and evidence suggests that BP significantly influences the polarization of macrophage M1 and M2 phenotypes. This review aims to present the regulatory effects and underlying mechanisms of 2D BP on macrophage polarization in the immune microenvironment. It highlights the ability of BP to systematically modulate the inflammatory environment and to facilitate the metabolic reprogramming of macrophages. The review concludes with a discussion of the potential applications and limitations of BP nanomaterials in the field of BTE.

Keywords: osteoimmune microenvironment, macrophage metabolic reprogramming, cell interaction, bone remodeling

Introduction

Among various systemic diseases, bone tissue defects and deformities resulting from factors such as trauma, tumors, and inflammation, along with the functional impairments they cause, have always been a significant challenge within the field of clinical medicine. Bone defects can lead to a reduction or complete loss of bone conduction and weight-bearing capabilities, significantly affecting the normal physiological functions of patients. The clinical management of bone defects presents difficulties in restoring bone integrity through anti-inflammatory repair methods alone. In addition, although autologous and allogeneic bone grafting are recognized as effective therapeutic approaches, they are still associated with risks such as immune rejection and suboptimal osseointegration outcomes.[1,2] Therefore, the development of BTE and regenerative medicine has become an inevitable trend in the clinical repair of bone defects. In recent years, with the wide application of cell culture technology, advanced nanomaterials, multifunctional hydrogels and 3D-printed scaffolds have gradually become the main materials for BTE.[3,4] These materials can mimic the native microenvironment

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and promote the recruitment, proliferation and differentiation of stem cells, as well as the construction of bone tissue structure.[5,6] Accordingly, it is an effective treatment for bone regeneration.

Dynamic regulation of the bone immune system occurs throughout the bone healing process, wherein macrophages assume a complex and nuanced role. For instance, bone macrophages adhering to the bone surface induce osteogenic differentiation and promote bone anabolism,[7] and these cells have the capacity to differentiate into pro-inflammatory M1 macrophages or anti-inflammatory M2 macrophages in response to both internal and external environmental stimuli, thereby influencing the homeostasis of the internal environment.[8,9] In the early stages of inflammation, M1-type macrophages play a crucial role in removing of pathogenic bacteria and necrotic tissue, thereby contributing to the maintenance of metabolic balance. Subsequently, these M1-type cells transition into M2-type macrophages, which are responsible for resolving inflammation and initiating tissue regeneration and repair.[9] However, an imbalance in the M1-M2 transition, coupled with the accumulation of inflammatory factors, can lead to osteoclast activation and inhibition of osteogenesis, ultimately impeding the bone healing process.[10] Macrophage populations respond to local environmental changes by adjusting the interconversion of M1 and M2, which significantly influences bone metabolism.[11,12] Consequently, macrophages emerge as critical targets in the context of bone regeneration facilitated by tissue engineering materials. Biomaterials enhance bone regeneration by improving the osteoimmune microenvironment and restoring the transformation balance of M1 and M2 phenotypes, thereby supporting a shift in the immune microenvironment from a pro-inflammatory to an anti-inflammatory state.[13,14]

As an emerging bio-nanomaterial, 2D BP exhibits a unique spatial structure and distinct physicochemical properties, including a high specific surface area, tunable band gap, and relatively facile surface functionalization. These characteristics allow it to demonstrate significant efficacy in drug delivery, photothermal therapy (PTT), and photodynamic therapy (PDT). Furthermore, since the primary degradation product of BP is non-toxic phosphate ions, the focus of clinical research has shifted from "immune evasion" to "immune interaction", further facilitating the application of BP in the field of tissue engineering.[15,16] Numerous studies have indicated that BP nanomaterials can effectively induce bone tissue regeneration. For instance, BP has been shown to activate the BMP-RUNX2 signaling pathway and the Wnt signaling pathway in stem cells, thereby effectively enhancing osteogenesis.[17–19] Additionally, there is evidence that BP possesses favorable bone immunomodulatory effects on bone,[20,21] that contribute to bone regeneration by modulating the overall immune microenvironment. However, to date, no comprehensive review has thoroughly examined the interactions between BP and the bone immune system. There remain significant gaps in our understanding of the bone immunomodulatory role of BP, particularly regarding the sequential regulation of the inflammatory microenvironment, the sequential activation of macrophages, and the effects on osteoclast differentiation by BP. These areas may represent significant avenues for research aimed at optimizing bone regeneration outcomes.

This paper provides a comprehensive review of the immunomodulatory effects of BP nanomaterials in the context of bone tissue regeneration. A substantial body of literature indicates that BP can enhance the inflammatory microenvironment of bone tissue, facilitate the transition of macrophages from the M1 phenotype to the M2 phenotype, and inhibit osteoclast differentiation, thereby promoting bone regeneration. Furthermore, BP has been shown to enhance neural tissue activity through immunomodulatory mechanisms, which further supports bone repair and regeneration. These findings establish a fundamental basis for the advanced application of BP in the field of BTE.

The Role of Macrophages in Bone Regeneration

In the physiological environment, bone homeostasis is dynamically regulated by the immune system. Within the immune microenvironment of bone, macrophages play a crucial role in facilitating bone anabolism or in activating osteoclasts leading to bone resorption, thereby regulating bone accumulation and influencing the prognosis of bone repair.[14,22]

Osteoblasts and osteoclasts play a critical role in the bone healing process, meticulously regulating bone remodeling through complex intercellular communication. And it has been demonstrated that the receptor activator of nuclear factor kappa-B ligand (RANKL)-receptor activator of NF-κB (RANK)-osteoprotegerin (OPG) signaling pathways are fundamental to bone metabolism. Various immune cells enhance RANKL expression to facilitate osteoclast differentiation and bone resorption, while OPG, mainly produced by osteoblasts, acts as a competitive antagonist to RANKL, and inhibits

osteoclastogenesis.[23] Furthermore, osteoblasts and osteoclasts secrete a number of active molecules that modulate each other's activity,[24] and thus work in concert to maintain the balance between bone regeneration and bone resorption.

Macrophages are capable of targeting osteoblast and osteoclast activities, thereby exerting various immunomodulatory effects on bone metabolism (as illustrated in Figure 1).[25] Among them, M1-type macrophages, induced by lipopolysaccharide (LPS) or interferon-gamma (IFN-γ) secrete pro-inflammatory factors that facilitate the clearance of pathogens.[26] However, under conditions of oxidative stress, M1 macrophages inhibit the proliferation and migration of pro-osteoblasts, leading to a reduction in bone mass.[27] Conversely, M2 macrophages induced by interleukin-4 (IL-4) and interleukin-13 (IL-13) secrete anti-inflammatory factors to construct an immune-friendly microenvironment,[26] and they can regulate the BMP2/SMAD signaling pathway in mesenchymal stem cells (MSCs) through paracrine mechanisms to enhance osteogenic differentiation and bone mass accumulation.[28,29] In summary, M1 and M2 phenotypic macrophages exert distinct immunomodulatory effects on bone homeostasis.

It's worth noting that while M1 macrophages typically contribute to bone resorption and osteogenic inhibition during chronic inflammation, they play a beneficial role in the early stages of injury by directing and initiating the bone healing process. For instance, M1 macrophages secrete tumor necrosis factor-alpha (TNF-α), which protects hematopoietic stem cells (HSCs) from necrotic apoptosis, while supporting HSC cell cycle activation and facilitating bone marrow regeneration.[30] In addition, M1 macrophages release activators that recruit bone marrow mesenchymal stem cells (BMMSCs)[31] and activate both the oncostatin M (OSM)-glycoprotein 130 (gp130) signaling complex and the bone
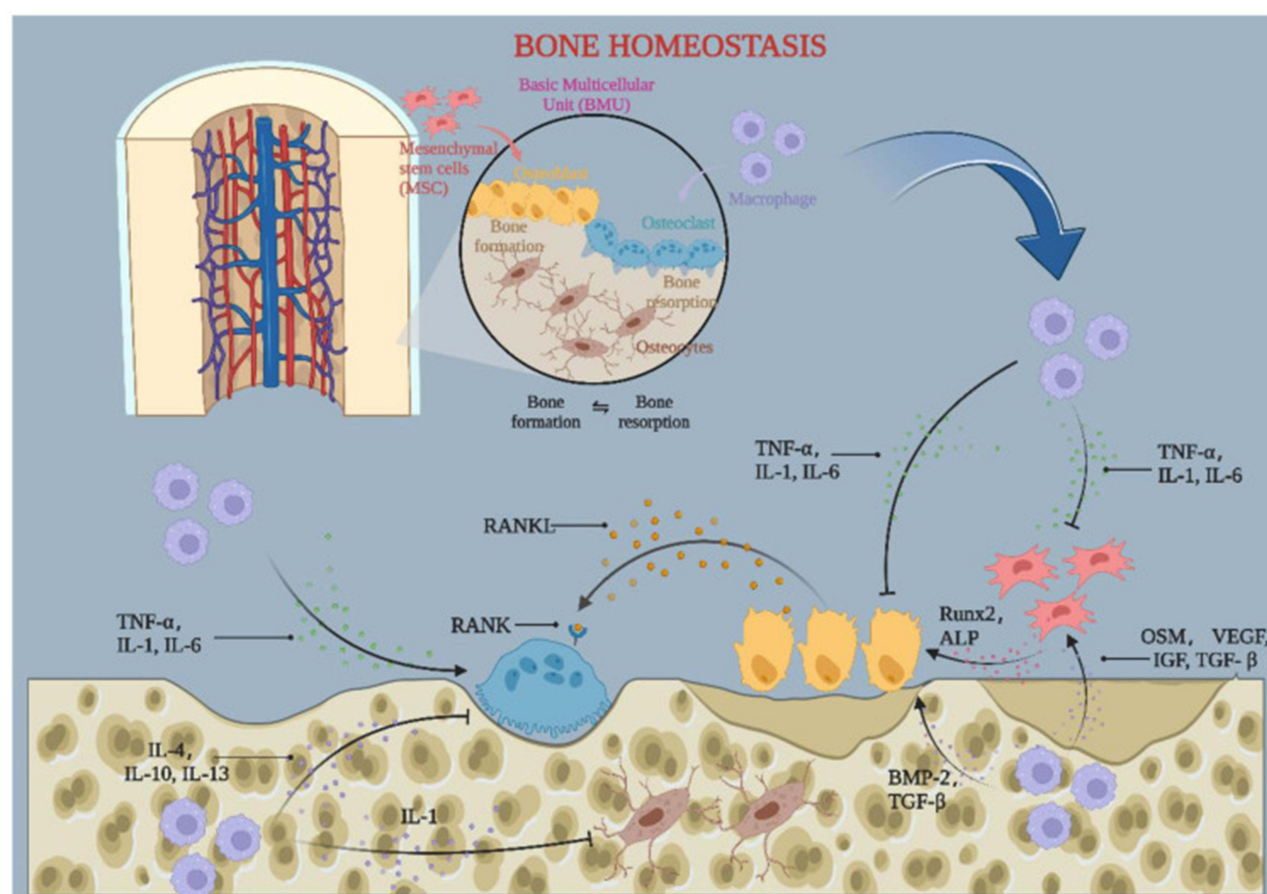

Figure 1 The role of macrophages in bone homeostasis. Macrophages secrete cytokines, including TNF-α, IL-1, and IL-6, which inhibit bone formation and promote bone resorption. They can also release cytokines such as IL-4, IL-10, and IL-13, inhibiting osteoclast differentiation. OSM, VEGF, IGF, TGF-β, and BMP-2 are secreted to stimulate osteoblast differentiation.
Notes: Reproduced from Hu Y, Huang J, Chen C, et al. Strategies of Macrophages to Maintain Bone Homeostasis and Promote Bone Repair: A Narrative Review. Journal of Functional Biomaterials. 2023; 14(1):18.© 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license.[25]

morphogenetic protein 6(BMP6)-SMAD pathway in stem cells, which are critical for early osteogenic differentiation.[22] Thus, it has been shown that M1 macrophages have a significant osteogenic induction effect on stem cells. Furthermore, it has been observed that an alkaline microenvironment induces M1 polarization and enhances the secretion of BMP6 and OSM, thereby promoting the expression of early osteogenic genes. It demonstrates a more pronounced positive bone immunomodulatory effect compared to M2 polarization induced in an acidic microenvironment.[32] In conclusion, sufficient evidence indicates that M1 macrophages are a critical driver of bone anabolism and occupy a unique position within the bone immune microenvironment.

As shown above, it is clear that M1 macrophages play a dual role in the process of bone healing: they facilitate bone anabolism while simultaneously inducing the occurrence of an early inflammatory environment. It's worth noting that the inflammatory response acts as a double-edged sword. While the initial inflammatory response is crucial for eliminating foreign bodies and bolstering tissue defenses, the sustained presence of M1 macrophages and the accompanying inflammatory cascade in damaged bone tissue can hinder the process of bone remodeling. Consequently, it is essential to address and mitigate this prolonged inflammatory response.

In conclusion, the activity of M1 and M2 type macrophages in the vicinity of bone tissue plays a significant role in the bone healing process. Therefore, the moderate modulation of M1/M2 polarization through the use of biomaterials represents an effective strategy to enhance bone regeneration.

BP Regulates the Inflammatory Microenvironment to Facilitate Bone Regeneration

The immune microenvironment plays a critical role in tissue injury, repair, and regeneration processes. Proper regulation of the immune environment is the cornerstone of bone regeneration. This is especially pertinent in the context of chronic inflammatory bone diseases, where the restoration and maintenance of bone immune homeostasis significantly influence disease prognosis. Extensive experimental data demonstrates that BP can systematically regulate the inflammatory environment and exert anti-inflammatory effects, thereby accelerating the bone remodeling process.[20,33,34]

Sequential Regulation of BP in the Pro-Inflammatory and Anti-Inflammatory Environment

The implantation of BP nanomaterials in the body inevitably induces an intrinsic immune response, but their excellent biocompatibility facilitates the regulation of inflammation, which is essential for the acceptance of BP by the host defense system.[35,36] It should also be clear that the acute pro-inflammatory and anti-inflammatory phases are not opposed to each other, but rather are a sequential process. The acute inflammatory phase prepares for the subsequent anti-inflammatory phase and is periodically regulated by the local environment, where the sequential regulation of inflammation and macrophage activation are crucial for tissue regeneration.[37–39] It has been demonstrated that BP is able to stimulate a certain degree of acute immune response and expedite the resolution of the inflammatory process, thus preventing tissue damage associated with prolonged chronic inflammation.[40,41]

Recent studies have demonstrated that BP can directly interact with macrophages, thereby activating early inflammatory responses and facilitating the transition from an inflammatory to a reparative environment. In the experiment, black phosphorus nanosheets (BPNSs) were co-cultured with resting macrophages (M0) and it was found that BPNSs transiently activated M0 cells, inducing a mild inflammatory response and progressively promoting an anti-inflammatory environment as the material naturally degrades. Simultaneously, the expression of the viral defense protein in macrophages was diminished, suggesting that BPNSs exhibit favorable biocompatibility.[42] Additionally, BP composite scaffolds can stimulate macrophages to express interleukin-33 (IL-33),[20] a crucial cytokine involved in the regulation of the immune response.[43] IL-33 plays a critical role in the systematic release of pro-inflammatory and anti-inflammatory factors in the local environment, as well as in the acceleration of osteogenic differentiation of BMMSCs.[20] Research on IL-33 has demonstrated that at an early stage, the IL-33/ST2 signaling axis activates the NF-κB and MAPK signaling pathways, thus inducing inflammatory responses.[44] However, as the concentration of IL-33 in the local environment increases, it exhibits an inhibitory effect on osteoclast differentiation and bone resorption. Additionally, it promotes the differentiation of M2-type macrophages and the expansion of

TIGIT+ regulatory T cells,[45] which collectively exert a more pronounced anti-inflammatory and reparative effect. Therefore, it can be concluded that BP facilitates the transition of injured tissues from the inflammatory phase to the repair phase by inducing the release of IL-33 from macrophages.

In addition, BP sequentially modulates the transformation of the immune environment by enhancing the metabolic activity of tissue cells. Research has demonstrated that BP can specifically activate peroxisome proliferator-activated receptor γ (PPARγ) to improve cellular metabolism and facilitate the transition out of the inflammatory phase.[40] PPARγ is a nuclear receptor found in various tissues and immune cells, playing a critical role in tissue glycolipid metabolism, inflammatory responses, and cell differentiation.[46] Notably, black phosphorus quantum dots (BPQDs) at a concentration of 0.1 mg/kg were observed to inhibit the PPARγ signaling pathway while activating the NF-κB signaling pathway, resulting in the infiltration of inflammatory factors. Conversely, BPQDs at a concentration of 1 mg/kg were found to activate PPARγ and the glycolytic pathway while inhibiting the NF-κB pathway, thereby mitigating tissue damage caused by inflammation.[40] Studies on PPARγ have revealed that crosstalk between the PPARγ and NF-κB pathways can reverse M1/M2 polarization and antagonize NF-κB signaling,[47] which partially elucidates the anti-inflammatory effects of BP. Furthermore, PPARγ is instrumental in maintaining the characteristic lipid metabolism of M2 macrophages,[48] and thus serves as a key mediator of the anti-inflammatory environment regulated by BP. Consequently, it is evident that upon entering the organism, BP can alleviate aspects of the acute inflammatory response by enhancing local tissue metabolism, thereby maintaining immune homeostasis.

Comprehensive analyses indicate that BP nanomaterials do not simply suppress local inflammatory responses during the repair of damaged tissue; rather, these materials initiate the inflammatory pathway early, establishing a controlled pro-inflammatory environment. This transient and moderate inflammatory response creates optimal conditions for subsequent tissue repair. Furthermore, BP possess the ability to regulate the transition from a pro-inflammatory to an anti-inflammatory environment according to a strict temporal sequence. The ultimate objective of this process is to establish an immune-friendly regenerative environment, thereby facilitating effective bone tissue regeneration.

BP Facilitates the Transformation of the Anti-Inflammatory Environment

In the treatment of bone tissue disorders, BP nanomaterials exhibit significant immunomodulatory benefits due to their antioxidant and slow degradation properties. Substantial evidence indicates that BP significantly enhances the release of numerous anti-inflammatory factors and bioactive molecules, thereby ameliorating the bone immune microenvironment.[21,49,50] For instance, during the wound-healing process following tooth extraction, BPNSs mitigate oxidative stress by scavenging reactive oxygen species (ROS) and support the regeneration of blood vessels and alveolar bone.[51] In the context of degenerative disc disease, BPQDs inhibit the acid-sensitive ion channel (ASIC)-mediated release of NLRP3 inflammatory vesicles and interleukin-1 beta (IL-1β), thereby fostering an anti-inflammatory environment and facilitating nucleus pulposus (NP) regeneration.[33] Furthermore, in the treatment of osteoarthritis (OA), BPNSs obstruct the secretion of metalloproteinases, specifically a disintegrin and metalloproteinase with thrombospondin motifs 5 (ADAMTS5), a disintegrin and metalloproteinase with thrombospondin motifs 1 (ADAMTS1), and matrix metalloproteinase 13 (MMP13),[34] while also directing the subchondral osteogenic process by modulating the inflammatory milieu. Additionally, in the treatment of hormonal osteonecrosis, periodontitis, and osteoporosis, BP has been shown to downregulate the pro-inflammatory factors such as IL-1β, TNF-α, interleukin-6 (IL-6), and inducible nitric oxide synthase (iNOS), while elevating the expression of anti-inflammatory factors such as interleukin-10 (IL-10), arginase-1 (Arg-1), and transforming growth factor-beta (TGF-β),[21,49,50] (as illustrated in Figure 2).[21] This regulatory mechanism ultimately establishes an immune microenvironment conducive to bone tissue repair. Meanwhile, in the context of bone tissue reconstruction following osteosarcoma resection, BP effectively stimulates the secretion of IL-10 and inhibits the expression of IL-6 and cyclooxygenase-2 (COX-2) in tumor cells,[52,53] thereby initiating the processes of bone repair and regeneration. In summary, it is evident that establishing an anti-inflammatory environment is essential for the processes of osteogenic differentiation and mineralization, with the immunomodulatory and reparative functions of BP playing a crucial role in bone remodeling.

A substantial body of literature indicates that BP possesses multiple mechanisms of action in modulating the anti-inflammatory environment. Firstly, as a potent photosensitizer, BP can synergistically facilitate the transformation of the anti-inflammatory milieu through photothermal therapy (PTT). Studies demonstrate that BP-based nanoplatforms can
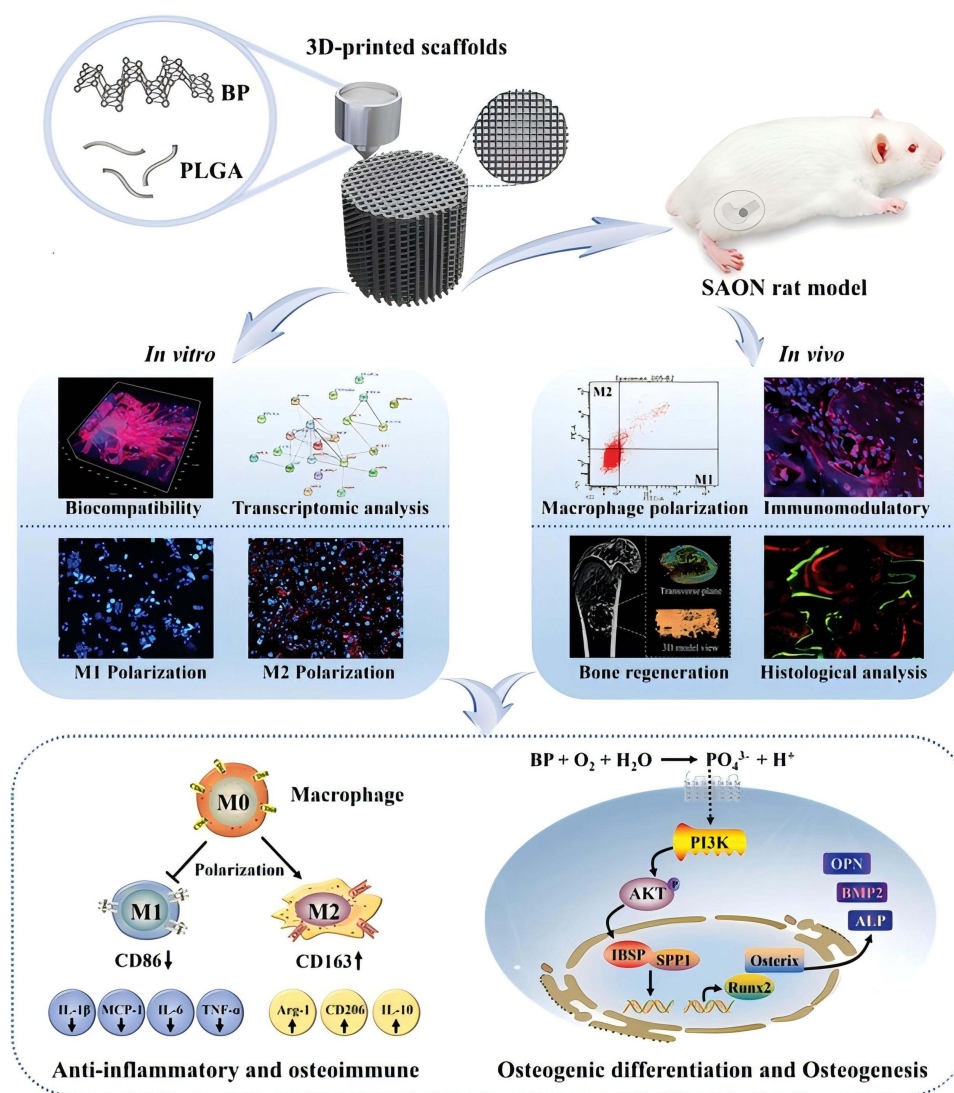
Figure 2 Mechanism of osteoimmune environment induced by BP degradation to accelerate bone regeneration. This study demonstrated that the PLGA/BP scaffold has osteogenic effects and bone immunomodulatory functions through in vitro and in vivo experiments.
Notes: Reproduced from Long J, Yao Z, Zhang W, et al. Regulation of Osteoimmune Microenvironment and Osteogenesis by 3D-Printed PLAG/black Phosphorus Scaffolds for Bone Regeneration. *Adv Sci* (Weinh). 2023 Oct;10(28):e2302539. © 2023 The Authors. Advanced Science published by Wiley-VCH GmbHThis is an open access article under the terms of the http://creativecommons.org/licenses/by/4.0/ License.[21]

inhibit the expression of TNF-α in wounds exposed to near-infrared (NIR) radiation. The reduction of TNF-α subsequently accelerates collagen fiber deposition and angiogenesis. Concurrently, NIR light enhances the inhibitory effects of BP on IL-1β and IL-6 secretion while promoting the induction of TGF-β and IL-10 secretion. The cytokine regulation by BP composites in the immune microenvironment is further detailed in Table 1.[41,54–57] In addition to the effects of PTT itself, it is noteworthy that PTT-activated heat shock protein 70 (Hsp70) exhibits beneficial immunomodulatory properties. Existing literature has shown that elevated intracellular levels of Hsp70 can inhibit NF-κB signal transduction and enhance the secretion of anti-inflammatory factors.[58] BP scaffolds have been shown to effectively increase the expression levels of HSP70 in periosteal-derived stem cells (PDSCs) and human bone marrow mesenchymal stem cells (hBMSCs) under NIR light irradiation,[59–61] with HSP70 further demonstrating significant immunomodulatory and osteogenic induction effects. Secondly, BP can enhance the metabolic activity of immune-responsive cells, thereby indirectly maintaining immune homeostasis. For instance, BPNSs can activate the JAK1-STAT1/3-OAS metabolic pathway in human umbilical vein endothelial cells (HUVECs),[42] which subsequently improves cytokine secretion and promotes vascular regeneration. Furthermore, BP has the ability to stabilize protein structures through its electrostatic adsorption

Table 1 Cytokine Regulation by BP Composites in the Immune Microenvironment

Materials	Platform	PTT/PDT	Therapy Mode	Effects	Ref
BPQDs	BP/CS/GelMA microspheres	–	Rat model of IVDD	Inhibit ASIC mediated release of NLRP3 inflammasomes and IL-1β	[33]
	BP/DOX/KIR/RBCm nanoparticles	-	Murine xenograft models of Hela tumour cells	Inhibit the secretion of IL-6 and TNF-α	[53]
	BP/PVA/Alg hydrogel	PTT	Rat model of MRSA infections in diabetic wounds	Inhibit the secretion of TNF-α, IL-1β and IL-6	[56]
	BP/Bi$_2$O$_3$/ε-PL/APBA-g-OCS/PVA hydrogel	PTT	Diabetic rats model of wound infection	Promote the secretion of IL-10 and TGF-β	[57]
	BP/EGCG conductive hydrogel	-	Rat model of SCI	Inhibit the secretion of IL-1, TNF-α, CCL3, CCL5 and CCL11	[67]
BPNSs	BP/Ag injectable hydrogel	PTT	Rat model of infected full-thickness wound defect	Inhibit the secretion of TNF-α	[41]
	BP solution	PTT	-	Promote the secretion of IL-10 Inhibit the secretion of IL-6 and COX-2	[52]
	BP/AS/MSN/PEG nano-spray	PTT	Rat model of infected full-thickness skin defect	Promote the secretion of IL-10 and TGF-β Inhibit the secretion of TNF-α	[54]
	BP/CA-CS/Odex hydrogel patch	PTT	Mouse model of infectious burn wound Mouse model of liver bleeding	Inhibit the expression of TNF-α, IL-1β, IL-6 and CD68	[55]
	BP/PLGA/V2C MXene bio-heterojunctions nanocatalytic membrane	SPT	Mouse model of infected full-thickness wound defect	Inhibit the NF-κB pathway	[68]
	BP/PEG-S2P peptide/RvD1	-	Mouse model of atherosclerosis	Inhibit the NF-κB pathway Inhibit the secretion of TNF, IL-1β and IL-6 Promote the secretion of IL-10 and TGFβ	[69]

Abbreviations: BPQD, black phosphorus quantum dots; IVDD, intervertebral disc degeneration; CS, chitosan nanoparticles; ASIC-3, acid-sensitive ion channel-3; DOX, doxorubicin; RBCm, Red blood cell membrane; KIR, Kirenol; PVA, polyvinyl alcohol; Alg, alginate sodium; Bi$_2$O$_3$, bismuth oxide; ε-PL, ε-polylysine; APBA-g-OCS, 3-aminophenylboronic acid modified oxidized chondroitin sulfate; SCI, spinal cord injury; EGCG, epigallocatechin-3-gallate; AS, Astragaloside IV; MSN, mesoporous silica nanoparticles; PEG, polyethylene glycol; CA-CS, catechol-modified chitosan; Odex, oxidized dextran; PLGA, poly(lactic-co-glycolic acid); SPT, Sono-piezo dynamic therapy; RvD1, resolvin D1.

properties, thereby mitigating the inflammatory burden on the body caused by the accumulation of abnormal proteins. For instance, BP has been shown to inhibit the deposition of β-amyloid (Aβ) and the abnormal folding of α-synuclein (α-syn) monomers, thus avoiding further inflammatory responses.[62,63] And BP stabilizes the structural integrity of angiotensin-converting enzyme 2 (ACE2),[64] which obstructs protein interactions and effectively prevents viral infections and immune disorders. Last but not least, BP serves as an excellent carrier role in combination with drugs and can effectively control the inflammatory response within the wound, achieving good anti-inflammatory and detoxifying effects.[65,66]

While BP promotes the transformation of the anti-inflammatory environment, it also enhances the survival of damaged tissue cells. Research has indicated that small quantities of BP can mitigate ROS-induced tissue damage and apoptosis,[70–72] (as illustrated in Figure 3).[72] This protective mechanism may be associated with the PDK1/AKT/14-3-3/ BAD and PI3K/Akt signaling pathways.[73,74] Additionally, BP regulates the cell cycle through the PDK1/AKT/GSK3 signaling pathway,[73] thereby facilitating tissue regeneration. Consequently, BP not only diminishes the detrimental effects of oxidative stress on the immune microenvironment but also activates the cell's intrinsic anti-apoptotic mechanisms, ultimately promoting tissue survival.

In conclusion, BP nanomaterials enhance the secretion of anti-inflammatory factors and bioactive molecules at the site of bone injury. They establish an immune-friendly microenvironment through intricate biological pathways, thereby creating optimal conditions for bone tissue regeneration.

BP Regulates Macrophages and Osteoclasts to Promote Bone Regeneration

Bone immune dysregulation represents a fundamental pathogenic factor in inflammatory bone diseases and persists throughout the progression of these conditions. Therefore, optimizing the bone immune environment is crucial for enhancing bone regeneration. Macrophages, as essential cellular components in the maintenance of bone immune homeostasis, have become a prominent focus of research in recent years. The subsequent section will provide
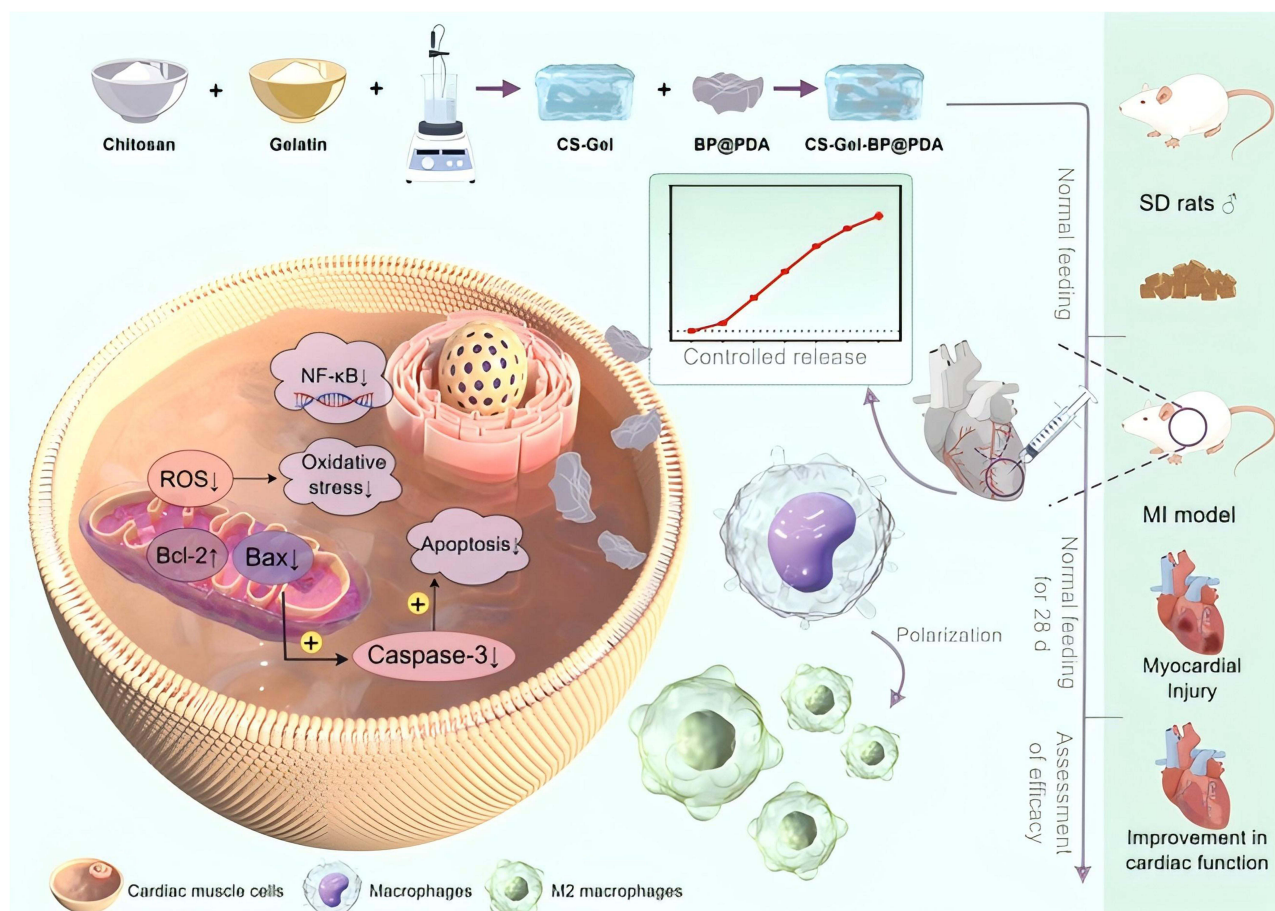
Figure 3 Schematic of the mechanism by which BP hydrogel motivates macrophage polarization and accelerates myocardial repair. CS-Gel-BP@PDA composite hydrogel can effectively attenuate oxidative stress, promote macrophage M2 phenotypic polarization, and facilitate the recovery of cardiac function.
Notes: Reproduced from Zhang J, Sun D, Liao Y, et al. Time-Released Black Phosphorus Hydrogel Accelerates Myocardial Repairing through Antioxidant and Motivates Macrophage Polarization Properties. *Biomater Res*. 2024 May 8;28:0029. Copyright © 2024 Jiahui Zhang et al.Exclusive licensee Korean Society for Biomaterials, Republic of Korea. No claim to original U.S. Government Works. Distributed under a Creative Commons Attribution License 4.0 (CC BY 4.0).[72]

a comprehensive discussion of the mechanisms underlying the immunomodulatory effects of BP on macrophages, ranging from passive immunity to active regulatory processes.

Pro-Inflammatory M1 Macrophages

When nanomaterials enter the bloodstream, they initially activate the body's passive immune response and rejection mechanisms, indicating enhanced immune defenses of the host. The acute inflammatory response at the injury site is characterized by the presence of M1 macrophages and neutrophils. Consequently, BP exhibited a transient mild activation effect on M1 macrophages during the early stages of inflammation. As some BP nanomaterials are swallowed into cells and others are gradually degraded in blood, BP exerts its antioxidant effects and inhibits cellular inflammatory signaling pathways. Therefore, BP demonstrates a significant inhibitory effect on M1 in inflammatory environments. The varying immunomodulatory effects of BP on M1 will be discussed in detail below.

BP Induces Intrinsic Immunity to Facilitate M1 Polarization

BP nanomaterials enter the bloodstream and initially activate the body's intrinsic immune response. Plasma proteins adsorb onto the surface of the nanomaterials, forming protein coronas. The black phosphorus corona complexes (BPCCs) interact with macrophage calmodulin, inducing calcium influx, which subsequently activates the NF-κB pathway within macrophages. This activation results in M1-type polarization[75] and a 2-4-fold increase in the secretion of proinflammatory factors.[76] Furthermore, an excess of intracellular ROS accelerates M1 production and inflammatory factors

infiltration.[77] Thus, the BPCCs formed by BP upon entering the bloodstream can interact with macrophages and induce M1-type polarization. Additionally, BP nanostructures can activate the integrin $\alpha v\beta 8$ signaling pathway on macrophage membranes, triggering an immune response;[78] however, they have minimal effects on the membrane, indicating that they can enhance macrophage immune response with low cytotoxicity. Furthermore, studies show that excessive levels of BPQDs result in ferroptosis in tissue cells,[79] which is characterized as a mode of inflammatory cell death. In this context, dead cells release chemokines such as CCL2 and CCL7, which subsequently trigger an acute inflammatory response marked by the presence of M1-type macrophages.[80] Consequently, the introduction of BP into the organism mildly activates M1 polarization and induces a shift in the inflammatory environment through various pathways.

It is worth noting that in the context of early inflammation, the generation of M1 macrophage populations is essential for strengthening host defenses. Beyond passively initiating the immune response, in certain instances, BP has the ability to actively stimulate M1 macrophages, thereby contributing to the maintenance of internal homeostasis. For instance, in the treatment of chronic osteomyelitis, BPNSs combined with iron oxides (Fe_3O_4) convert electromagnetic waves into thermal energy, resulting in a microwave thermal effect (MTE). MTE further activates the immune response and enhances the phagocytic activity of immune cells, thereby improving the overall bactericidal effect.[81] Furthermore, in the field of tumor therapy, BP can selectively target tumor tissues, facilitate the metabolic reprogramming of M2 to M1 macrophages, and induce tumor cell apoptosis.[82,83] Therefore, given the intricate relationship between macrophage activity and bone regeneration, the subsequent discussion will focus on the inhibitory role of M1 macrophages in chronic inflammation.

BP Improves the Inflammatory Environment and Inhibits M1 Polarization

In inflammatory environments, the antioxidant BP is capable of obstructing the inflammatory cascade and inhibiting the polarization of pro-inflammatory M1 macrophages. For instance, in models of osteoarthritis, periodontitis, and myocardial injury, BPNSs effectively scavenge excess ROS and inhibit the expression of M1-related cytokines, ultimately inducing the metabolic reprogramming of macrophages.[34,49,84] Furthermore, BP has been shown to directly inhibit the NF-κB signaling pathway, a critical inflammatory pathway in M1 macrophages that is essential for maintaining immune homeostasis.[68,69] And it is also noteworthy that PPARγ can act as a negative regulator of pro-inflammatory responses and M1 activation. Moderate concentrations of BP indirectly inhibit M1 production by targeting PPARγ activation.[40,85] However, aberrant activation of PPARγ specifically promotes osteoclast differentiation while inhibiting osteoblast differentiation, which is closely associated with bone resorption in inflammatory bone diseases.[85] Based on the theme of bone immunomodulation by BP, this aspect will not be further discussed here. In summary, BP, as a natural antioxidant that scavenges free oxygen radicals and limits the polarization of M1-type macrophages, plays a significant role in the regulation of inflammatory responses.

Furthermore, BP has the capacity to eliminate M1-associated inflammatory cells and indirectly inhibit M1 activation. Research has demonstrated that the local inflammatory milieu in rheumatoid arthritis (RA) fosters the production of invasive synovial fibroblasts (SFs),[86] while elevated levels of aerobic glycolysis support their increased proliferative activity.[87] SFs further interact with synovial macrophages (SMs) to enhance the metabolism of SM cells and increase their expression of pro-inflammatory factors.[88] This synergistic interaction exacerbates the inflammatory response within the synovium. In the context of RA treatment, BP delivers substantial amounts of ROS to the inflamed joints under NIR, thereby eliminating excess proliferative synovial tissue.[89] Additionally, BP induces apoptosis or necrosis of SFs in the joint cavity of RA,[36] and promotes the M1-M2 phenotype transition in local macrophages,[90] which significantly mitigates the inflammatory response and preserves metabolic homeostasis. This mechanism may be associated with the activation of the P53 apoptotic pathway in inflammatory cells by BP.[91,92]

The immunomodulatory effects of BP can be further enhanced by surface modification or drug loading. For instance, the surface modification of BP with titanium sulfonate ligand (TiL4) significantly reduces the secretion of pro-inflammatory factors by macrophages and inhibits the infiltration of peripheral neutrophils.[35] Furthermore, the combination of BP with Icaritin (ICT) or the incorporation of procyanidin B2 (OPC B2) can effectively manage M1-type acute inflammation and promote the conversion of M1 (CD86+) macrophages to M2 (CD206+) macrophages,[93,94] thereby contributing to the resolution of inflammation.

The biocompatibility of BP nanomaterials has been demonstrated by various studies. Assessment of apoptotic markers following the co-culture of BP with cells indicated that BP did not exhibit significant cytotoxicity.[63,95]

Although naked BP (BPQDs) resulted in a transient increase in the levels of peripheral neutrophils, pro-inflammatory factors, and inflammatory macrophages in the target organs of mice, the overall inflammatory response was found to be reversible, gradually returning to baseline levels over time.[35,36] Furthermore, research has demonstrated that BPQDs can be effectively eliminated from the body via renal excretion,[96] without causing damage to vital organs or inducing inflammatory lesions.[68,96] These findings support the potential application of BPQDs in biomedical fields.

Anti-Inflammatory M2 Macrophages

The Direct Role of BP in Promoting M2 Polarization

In the dynamic regulation of the immune system, anti-inflammatory M2 macrophages typically emerge later in the immune response and play a crucial role in facilitating bone tissue repair. A substantial body of literature indicates that BP can target macrophages to induce M2 polarization and remodel the anti-inflammatory environment. For instance, in models of periodontitis and skull defects, BPNSs promote the reprogramming of M1 to M2 macrophages and enhance the secretion of M2-specific anti-inflammatory factors,[19,49,97,98] (as illustrated in Figure 4),[49] which contributes to improved disease prognosis. In the treatment of osteoporosis, BP facilitates the M1 to M2 transition while simultaneously inhibiting the differentiation of macrophage-derived osteoclasts,[50] thereby guiding bone regeneration through general immunomodulation. In the treatment of infected and diabetic wounds, BP administration has been observed to drive the production of M2 (CD206) macrophages. Subsequently, these M2 macrophages release various active factors that facilitate angiogenesis and tissue healing.[19,99–101] The promoting effect of BP composites on macrophage phenotypic transformation is further described in Table 2. Consequently, the polarization of M2 macrophages induced by BP is critical for bone regeneration. Furthermore, the PI3K-Akt signaling pathway has been shown to be a crucial pathway for M2 polarization,[102] and the downstream factor mechanistic target of rapamycin complex 2 (mTORC2) serves as a marker for M2 cells.[103] Evidence suggests that the intracellular PI3K/Akt signaling pathway activated by BP is intricately linked to the metabolic reprogramming between M1 and M2 macrophages.[21,104,105] Therefore, it is hypothesized that BP may enhance M2 polarization via the PI3K-Akt-mTORC2 signaling axis, thereby modulating the bone immune micro-environment. However, further validation through extensive experimentation is required.
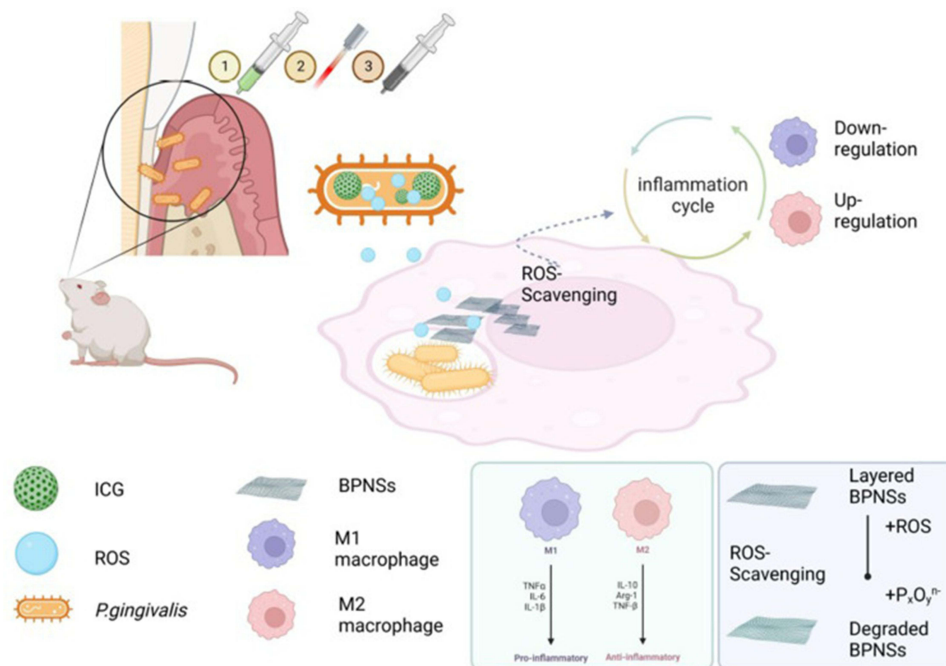

Figure 4 BPNSs promote the metabolic reprogramming of macrophages in local periodontal inflammation. Administration of BPNS during aPDT-mediated periodontitis treatment protects against ROS-induced oxidative damage and polarizes pro-inflammatory M1 macrophages into anti-inflammatory M2 macrophages.
Notes: Reproduced from Li X, Ren S, Song L, et al. Combined Black Phosphorus Nanosheets with ICG/aPDT is an Effective Anti-Inflammatory Treatment for Periodontal Disorders. *Int J Nanomedicine*. 2023 Feb 15;18:813-827. © 2023 Li et al.This work is published and licensed by Dove Medical Press Limited. The full terms of this license are available at https://www.dovepress.com/terms.php and incorporate the Creative Commons Attribution – Non Commercial (unported, v3.0) License.[49]

Table 2 Promotional Effect of BP Composites on Macrophage Phenotype Transformation

Materials	Platform	PTT/ PDT	Therapy Mode	Functions	Ref
BPNSs	BP/PLGA 3D printed scaffolds	–	Rat model of SAON	Promote M2-type polarization Activate the PI3K/Akt pathway	[21]
	BP GelMA hydrogel	-	Rat model of full-thickness wound defect	Drive M1-M2 phenotypic transformation	[42]
	BP/ICG solution	aPDT	Rat model of periodontal disease	Drive M1-M2 phenotypic transformation	[49]
	BP/CP nanoparticles	-	Mouse model of osteoporosis	Drive M1-M2 phenotypic transformation Inhibit osteoclast differentiation	[50]
	CS/BP/PDA Gel	PTT	Rat model of MI	Promote M2-type polarization	[72]
	BP/EGCG hydrogel	-	Mouse model of SCI	Inhibit microglia and macrophages activation	[73]
	BP/albumin scaffold cardiac patch	PTT	Rat model of MI	Promote M2-type polarization	[84]
	BP/NBP injectable hydrogel	PTT	Rat model of skull defect	Drive M1-M2 phenotypic transformation	[97]
	BP/TA-Mg/SFMA hydrogel	-	Rat model of skull defect	Drive M1-M2 phenotypic transformation	[98]
	BP/oDex/ZnO/Gel hydrogel	PTT	Rat model of infected full-thickness wound defect	Promote M2-type polarization	[99]
	BP/QCS/PLLA/Hb nanofibers	PTT	Diabetic mouse model of skin wound	Drive M1-M2 phenotypic transformation	[100]
	BP/PEG/CS/HA/bFGF microspheres	PTT	Rat model of full-thickness wound defect	Drive M1-M2 phenotypic transformation	[101]
	HACC-BP/PLGA/Rg 1/Gel asymmetric membrane	PTT	Rat model of full-thickness wound defect	Promote M2-type polarization Activate PI3K/Akt pathway	[104]
	BP/HAMA/ZIF-8/GelMA hydrogel	PTT	Rat model of Cranial defect	Promote M2-type polarization Activate PI3K/Akt pathway	[105]
	BP/CS/DFO hydrogel	PTT	Rat model of full-thickness wound	Upregulate IL-19 expression Promote M2-type polarization	[106]

Abbreviations: SAON, steroid-associated osteonecrosis; PLGA, poly(lactic-co-glycolic acid); aPDT, antibacterial photodynamic treatment; CP, copper peroxide; MI, myocardial infarction; CS, chitosan; PDA, polydopamine; EGCG, epigallocatechin-3-gallate; SCI, spinal cord injury; NBP, dl-3-n-butylphthalide; TA, tannic acid; SFMA, methacrylate silk fibroin; oDex, oxidized dextran; PLLA, poly-l-lactide; QCS, quaternized chitosan; Hb, hemoglobin; PEG, polyethylene glycol; HA, hyaluronic acid; bFGF, basic fibroblast growth factor; Rg1, ginsenoside Rg1; ZIF-8, zeolitic imidazolate framework-8; HAMA, methacrylate-modified hyaluronic acid; DFO, deferoxamine mesylate.

Furthermore, it has been suggested that BP facilitates M2 polarization by activating the cellular autophagy pathway. Cellular autophagy, which occurs at basal levels in all tissues, plays a crucial role in alleviating oxidative stress and exhibiting anti-apoptotic effects.[107] Previous studies have indicated that the autophagy pathway is involved in IL-10-induced M2 polarization.[108,109] BP has been shown to induce cellular autophagy, as evidenced by the induction of autophagosome formation, upregulation of Beclin-1 gene expression and promotion of the conversion from LC3-I to LC3-II.[110,111] The mechanism involved may be related to the activation of the PERK-ATF4-CHOP pathway.[111] And BPQDs can activate macrophage autophagy to reduce the inflammatory response and promote lipid metabolism.[112] Therefore, it can be posited that the process of BP-induced M2 polarization may be partially dependent on the activation of autophagy. However, the precise mechanisms involved require further investigation.

The Indirect Role of BP in Promoting M2 Polarization

In addition to the direct induction of M2 polarization, the indirect effects of BP on macrophages deserve considerable attention. First, PTT has been demonstrated to upregulate cytosolic integrin α4 and activate the PI3K-Akt1 signaling pathway, which subsequently facilitates M2 polarization.[28] The induction of M2 polarization by BP is markedly enhanced when combined with PTT,[93,113] ultimately accelerating bone remodeling. Second, electrical stimulation serves

as an effective promoter of M2 polarization. BP-based electroactive hydrogels, when supported by electrical stimulation, can improve the immune microenvironment and inhibit the activation of inflammatory cells,[67] thereby promoting M2 polarization, (as illustrated in Figure 5a–c)[114] and aiding in the repair of tissue damage. Consequently, the exceptional photothermal and conductive properties of BP are advantageous for establishing an anti-inflammatory microenvironment and promoting tissue regeneration.

Furthermore, BP has the ability to activate the intracellular antioxidant pathway, thereby inducing the production of M2 macrophages. The Nrf2/Keap pathway functions as a critical defense mechanism for cells, facilitating the maintenance of homeostasis and playing a significant role in the survival of tissue cells. Research has demonstrated that the Nrf2/Keap pathway promotes the differentiation of M2 macrophages[115] while simultaneously antagonizing NF-κB receptors and activating PPARγ receptors,[116] thereby indirectly modulating the immune microenvironment. Evidence indicates that BP activates the intracellular Nrf2 pathway,[117] which contributes to the maintenance of tissue homeostasis and results in the upregulation of Nrf2 protein and heme oxygenase-1 (HO-1) expression. This enhancement in tissue
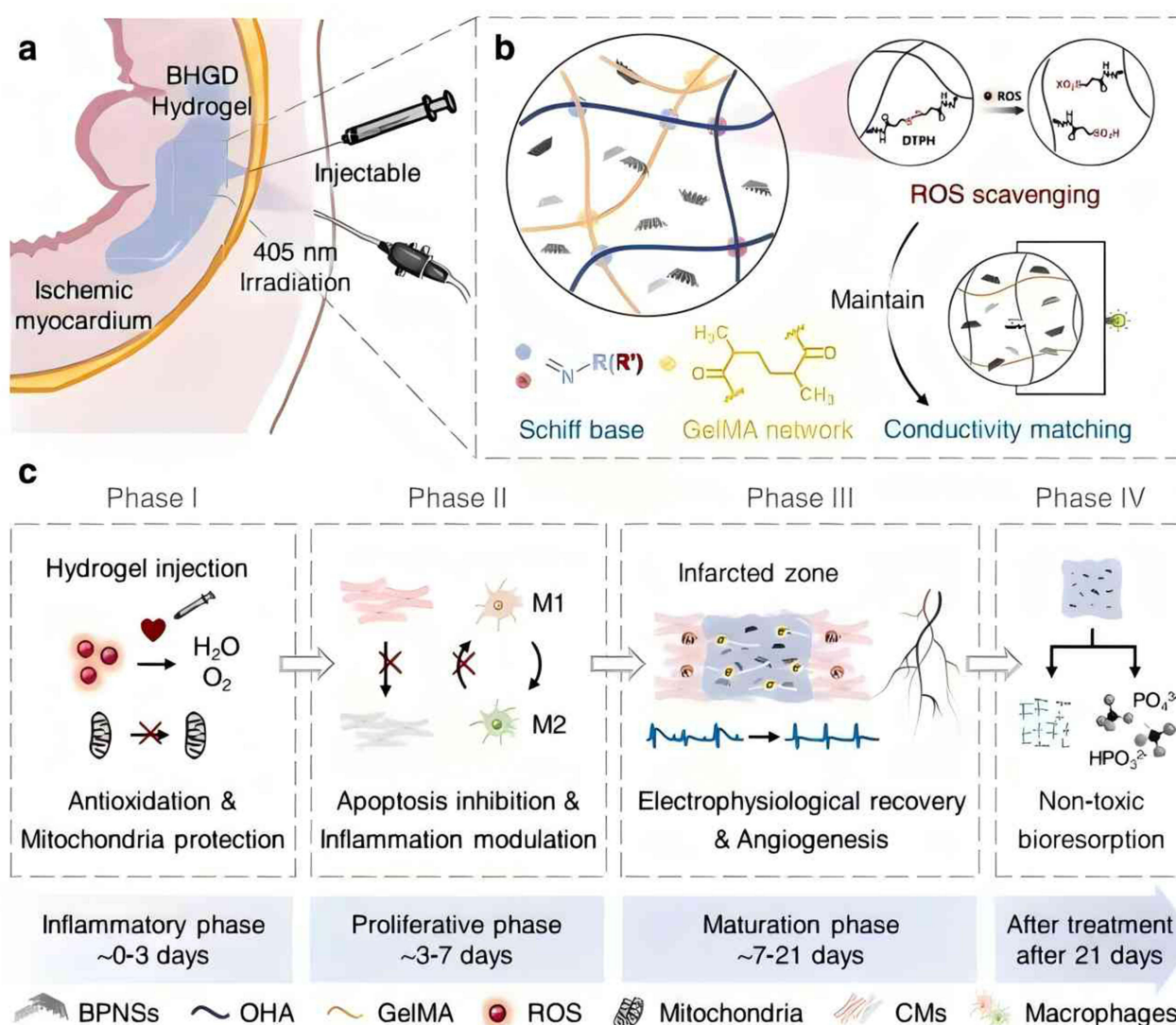

Figure 5 Electroactive BP hydrogels inhibit cellular oxidative stress damage and promote macrophage M2 polarization.(**a**) Injection process of the BHGD hydrogel into the ischemic myocardium. (**b**) Preparation and properties of the BHGD hydrogel. (**c**) Modulation of the MI microenvironment and bioresorption mechanism of the BHGD hydrogel in vivo.
Notes: Reproduced from Qiu Y, Yu C, Yue Z, et al. Chronological-programmed black phosphorus hydrogel for responsive modulation of the pathological microenvironment in myocardial infarction. *ACS Appl Mater Interfaces*. 2024. Copyright © 2024 American Chemical Society.[114]

metabolic activity ultimately fosters vascularization and neural regeneration.[95,118] Concurrently, the increase in ROS also triggers the activation of the Nrf2/Keap pathway, which enhances cellular adaptation to oxidative stress.[119] In conclusion, BP effectively activates the intracellular Nrf2/Keap pathway in response to oxidative stress, facilitating the establishment of an anti-inflammatory environment characterized by M2-type macrophages.

Furthermore, the metabolites of BP can indirectly enhance M2 polarization. The exceptional bioelectrical conductivity of BP increases the intracellular concentration of free Ca^{2+}[94] and promotes the formation of calcium phosphate through in situ phosphorus-driven calcium extraction.[120] Calcium phosphate nanoparticles interact with the cell surface integrin β1, activating the PI3K/Akt signaling pathway, which induces M2 polarization and subsequent osteoblast differentiation.[121] Consequently, both BP monomers and the metabolite calcium phosphate are capable of promoting M2 polarization, thereby accelerating the process of bone regeneration.

Finally, BP can serve as a drug reservoir to facilitate the conversion of M1 to M2 macrophages. For instance, the co-loading of graphene oxide (GO) layers and BPNSs with IL-4 can alleviate local inflammation while driving M2 polarization.[122] Furthermore, the combination of carbon nanotubes (sdCNTs), BPNSs and dexamethasone (DEX) is used to induce osteogenesis, while regulating the transition to an M2 polarization and an anti-inflammatory environment,[123] ultimately accelerating vascularization and osseointegration. Therefore, it is evident that BP can enhance the efficacy of bone immunomodulatory drugs, helping them to achieve their optimal therapeutic effects.

It is essential to recognize that the role of M2 macrophages cannot be generalized in a single statement. Regenerative repair associated with excessive M2 polarization may also contribute to the development of certain diseases. For instance, after skeletal muscle injury, TGFβ1-producing macrophages are directly linked to the aberrant differentiation of progenitor cells, which ultimately leading to heterotopic ossification (HO).[124] Notably, BP has been shown to inhibit M2 polarization and preserve metabolic homeostasis under pathological conditions. For example, after acute ocular surface injury, BPQDs scavenge ROS and impede the production of intracellular neutrophil extracellular traps (NETs), thereby mitigating pathological corneal fibrosis associated with M2 polarization.[125,126] In conclusion, BP does not exert a singular regulatory effect on macrophages; rather, it dynamically modulates the M1/M2 ratio and contributes to the maintenance of immune system homeostasis.

In summary, the immunomodulatory strategy of BP includes several key aspects. First, BP activates the body's innate immune system. As the material is gradually degraded in vivo, BP effectively eliminates ROS that accumulate in inflammatory environments. This process inhibits the activation of inflammatory signaling pathways and the production of M1-type macrophages, thereby mitigating the inflammatory response at the site of infection. Second, BP promotes the production of M2-type macrophages, particularly when combined with PTT and electrical stimulation, ultimately fostering an immune environment conducive to bone tissue repair.

Osteoclasts

Monocytes and macrophages serve as the primary source of osteoclast differentiation. The overactivation of osteoclasts is linked to a reduction in bone mass[85] and plays a pivotal role in inflammation-induced bone loss.[127,128] Furthermore, osteoclasts can induce H-type angiogenesis, which enhances the communication and interaction between immune cells and tissues.[129] Consequently, targeted modulation of osteoclasts represents a significant strategy for maintaining bone immune homeostasis and metabolic balance.

In addition to its role in regulating macrophage polarization, BP can also directly influence osteoclast differentiation, thereby affecting osseointegration. For instance, oxidized black phosphorus nanosheets (oBPNSs) have been shown to inhibit the phosphorylation of p38 mitogen-activated protein kinase (MAPK) in RAW264.7 cells and subsequently downregulate the NF-κB pathway and the expression of the NFATc1 gene. This effect ultimately leads to a reduction in RANKL-induced osteoclast differentiation.[130] Additionally, the degradation product of BP, inorganic phosphate (Pi), exerts a negative feedback regulation on RANK-RANKL signaling, downregulates osteoclast gene expression, and antagonizes the activity of the NF-κB pathway.[131] Furthermore, BP stimulates the secretion of IL-33 by macrophages, and elevated levels of IL-33 have been found to inhibit osteoclast formation while promoting environmental changes conducive to bone regeneration.[20] In conclusion, osteoclast differentiation is intricately regulated by BP, a process that is crucial for enhancing the bone immune environment and facilitating bone mass accumulation. Moreover, both the

reduced state and the oxidative degradation products of BP can modulate the bone immune environment in a manner that promotes beneficial bone regeneration, demonstrating the favorable biocompatibility and biosafety of the nanomaterials, which establishes a foundation for their advanced applications in biomedical fields.

Conclusion and Future Perspectives

Over the past few years, research on 2D nanomaterials has progressively deepened, leading to a paradigm shift in the field of BTE. This shift has transitioned from a singular emphasis on the osteogenic properties of materials to a more comprehensive investigation of their effects on the bone immune environment. BPNSs, as a novel class of nanomaterials, have exhibited significant potential in promoting bone regeneration and modulating immune responses within the bone. A substantial body of research has demonstrated that BPNSs can interact with the bone immune microenvironment to elicit a specific immune response. Furthermore, the inherent antioxidant properties of BP contribute to the establishment of an anti-inflammatory repair environment and facilitate targeted alterations in macrophage phenotypes, ultimately enhancing bone regeneration.

Recent studies have demonstrated that BP can also enhance the immune microenvironment surrounding nerves and indirectly facilitate innervated bone regeneration, thus filling a gap in its regulatory mechanisms in bone remodeling. For instance, magnesium ion-modified BPNSs (BP@Mg) have been shown to diminish the accumulation of inflammatory cells in infected environments, improve the functionality of Schwann cells (SC), and promote innervated bone regeneration.[132,133] Meanwhile, BPNSs have been shown to activate the Nrf2 pathway in neural progenitor cells (NPCs), which directly modulates intracellular oxidative stress responses and promotes neural differentiation.[117] Additionally, Studies have shown BP-mediated electrical stimulation effectively promotes the transformation of Schwann cells into an active repair phenotype and supports nerve-induced osteogenesis.[134] In conclusion, there is considerable evidence that BP guides nerve regeneration during the early stages of bone repair through immune modulation.

Despite the established anti-inflammatory and pro-repair properties of BP, several critical challenges remain that must be addressed. Current research trends predominantly emphasize the role of BP-induced polarization of M2-type macrophages in bone regeneration. However, it is essential to acknowledge that during the actual bone healing process, both M1- and M2-type macrophages work synergistically to enhance stem cell recruitment, promote osteoblastic activity, and facilitate matrix mineralization, ultimately leading to effective bone remodeling. Consequently, it is imperative to elucidate the differential regulatory effects of BP on M1 and M2 macrophages at all stages of bone healing, as well as their respective contributions to bone remodeling, in order to develop optimal strategies for bone regeneration. Furthermore, the bone immune microenvironment is a dynamic and self-regulating ecosystem in which a diverse array of immune cells collaborate to maintain environmental homeostasis. Significant gaps remain in our understanding of the effects of BP on other immune cell types, including neutrophils, mast cells, monocytes, dendritic cells, and lymphocytes, as well as their collective regulation of osteogenic activity, which warrants further investigation.

Furthermore, the mechanisms by which BP nanomaterials influence immune cell metabolism remain inadequately understood. Research indicates that the regulation of bone immune homeostasis is fundamentally linked to the modulation of metabolic pathways in immune cells. Specifically, M0 and M2 macrophages predominantly utilize the tricarboxylic acid cycle and oxidative phosphorylation to sustain their cellular functions, whereas M1 macrophages exhibit a greater reliance on glycolysis, which facilitates a prompt and robust inflammatory response.[26,135] Additionally, the hypermetabolic profile characteristic of M1 macrophages is closely associated with a refractory inflammatory environment.[103] Evidence suggests that BP nanomaterials specifically target the respiratory metabolism of immune cells, promoting M2 polarization in macrophages[21,104] and enhancing the immune response of natural killer (NK) cells.[136] Consequently, the regulation of immune cell metabolism to induce alterations in cellular phenotype and function, thereby fostering a favourable immune microenvironment, may represent a promising avenue for future research into the role of BP in bone immunomodulation.

Finally, the biological application limitations of BP nanomaterials cannot be overlooked. These limitations include a rapid degradation rate in vivo, cytotoxicity to specific cell types, and the properties of BP nanomaterials—such as surface morphology, surface charge, porosity, wettability, and particle size—that significantly influence their interactions with immune cells. Therefore, only through appropriate surface and chemical modifications can the degradation of BP be delayed, and protein adsorption and subsequent immune responses be precisely regulated, thereby optimizing bone regeneration. However, current research on the combined applications of multiple biomaterials remains limited. It is still to be determined which dimensions of BPNSs are most

effective for bone immunomodulation and regeneration, how to align the optimal timing for immune modulation with the degradation rate of materials through surface modification, and the impact of biomaterial modifications on immune cell activities. Consequently, future research should prioritize the joint application of BP and various nanomaterials to advance the development of bone tissue engineering by leveraging the complementary strengths of multiple materials.

Data Sharing Statement

Data availability is not applicable to this article as no new data were created or analyzed in this study.

Acknowledgments

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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