The crosstalk between macrophages and bone marrow mesenchymal stem cells in bone healing

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Abstract
Bone injury plagues millions of patients worldwide every year, and it demands a heavy portion of expense from the public medical insurance system. At present, orthopedists think that autologous bone transplantation is the gold standard for treating large-scale bone defects. However, this method has significant limitations, which means that parts of patients cannot obtain a satisfactory prognosis. Therefore, a basic study on new therapeutic methods is urgently needed. The in-depth research on crosstalk between macrophages (Mφs) and bone marrow mesenchymal stem cells (BMSCs) suggests that there is a close relationship between inflammation and regeneration. The in-depth understanding of the crosstalk between Mφs and BMSCs is helpful to amplify the efficacy of stem cell-based treatment for bone injury. Only in the suitable inflammatory microenvironment can the damaged tissues containing stem cells obtain satisfactory healing outcomes. The excessive tissue inflammation and lack of stem cells make the transplantation of biomaterials necessary. We can expect that the crosstalk between Mφs and BMSCs and biomaterials will become the mainstream to explore new methods for bone injury in the future. This review mainly summarizes the research on the crosstalk between Mφs and BMSCs and also briefly describes the effects of biomaterials and aging on cell transplantation therapy.

Keywords: BMSCs, Macrophages, Inflammation, Tissue regeneration, Biomaterial

Introduction
Bones consist of 65% inorganic substance and 35% organic substance and also contain almost 99% calcium and 85% phosphorus in the whole body [1, 2]. Outer cortical bone provides considerable strength under the existence of hydroxyapatite, while inner cancellous bone provides nutrition and hematopoietic function for bones [1]. There are about 20 million people around the world to suffer from bone fractures which are mainly caused by external force or tissue lesions every year, and it brings heavy economic burdens to the public medical insurance system [3, 4]. Currently, large-scale bone defects need challenging surgical intervention to repair, such as autologous bone transplantation (the gold standard) [5, 6]. Maxillofacial surgeons usually take parts of bones from patients’ fibula to replace their diseased mandibles. However, it must be pointed out that autologous tissue transplantation will bring a series of complications to patients, which means not all patients can be guaranteed a good prognosis. Therefore, it is necessary to explore new methods for orthopedists to treat a large population with bone fractures.

Primary healing (direct method) and secondary healing (indirect method) are two healing modes for bone injury [7]. This kind of healing model is different from that of other tissues, due to the fact that there are no scar

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tissues formed in the whole process [8]. As the most ideal healing model for injured bones, there are three steps in secondary healing (hematoma formation, primary callus formation, and bone mineralization). The interactions among cells, signaling pathways, cytokines, and extracellular matrix are thought to be key factors to determine the prognosis of bone healing. Among the kinds of functional cells in bone healing, Mψs and BMSCs are unique and critical [9, 10]. In the resting state, osteoblasts differentiated from BMSCs and osteoclasts differentiated from mononuclear-Mψs keep the formation and absorption balance in bones [11].

Mψs consist of resident Mψs derived from the yolk sac in the embryonic stage and hematopoietically derived Mψs derived from bone marrow (Fig. 1) [12]. Resident Mψs mainly existed in alveoli (alveolar macrophages), brain (glial cells), abdominal cavity (peritoneal macrophages), etc., and they have self-renewal capacity to keep cell population stable for life [13–15]. Hematopoietically derived Mψs can be harvested from circulating monocytes, and mediate immune responses in target areas after being transported through blood [16]. Mψs have the ability to eliminate cellular debris to keep the microenvironment stable by activating suitable inflammatory cascade reaction [17–20]. Recent research has shown that Mψs would polarize toward pro-inflammatory phenotype and anti-inflammatory phenotype in the specific microenvironment [21, 22]. Wan et al. reviewed that the selective depletion of Mψs induced in vivo caused long-term wound nonunion [23]. It was suggested that Mψs not only act as inflammatory cells to eliminate necrosis components, but also as functional cells to promote tissue regeneration.

Like adipose stem cells (ASCs) in adipose tissues [24] and satellite cells in skeletal muscles [25], BMSC is a type of stem cell in bone tissues [26]. They were first isolated from human body 40 years ago and could self-proliferate and differentiate in vitro [27]. Flow cytometry tests show that BMSCs are CD73+/CD90+/CD105+/CD11b−/CD14−/CD34−/CD45−/CD19−/CD79a−/human leukocyte antigen-DR− (HLA-DR−) [28, 29]. As progenitor cells for osteogenesis, BMSCs are able to counteract...
considerable degrees of bone non-pathological absorption to maintain bone structure integrity [11]. In recent years, BMSCs derived from bone marrow have been proved to exist around peripheral blood vessels and directional differentiation in targeted damaged tissues by blood transportation [26, 30–33]. Some scientists thought that cell niches in blood vessels could enhance the migration of BMSCs, which is beneficial to tissue regeneration [34].

Bone regeneration needs the cooperation of multiple systems. Among the systems that we knew, bone system and immune system are gradually considered to be vital in bone healing, due to the fact that they share many molecular regulatory networks, which creates an emerging discipline called osteoimmunology [35–37]. In conclusion, osteoimmunology studies the interactions between immune cells and bone cells in bone regeneration. In this review, we systematically retrospect the research on the crosstalk between BMSCs and Mφs in bone healing in recent years and aim to provide new ideas for further research on bone regeneration based on cell transplantation.

**The function of Mφs in bone healing and its regulatory role on BMSCs osteogenesis**

Mφs have been found to participate in lots of important life processes to maintain tissue homeostasis, including infection and regeneration [38, 39]. Since a long time ago, scientists generally believed that Mφs were differentiated from monocytes derived from bone marrow and spread to target organs through the circulatory system [40–42], which is based on the differentiation of leukocytes in blood into Mφs and dendritic cells in vivo. However, recent studies have found that parts of Mφs were derived from embryonic yolk sac cells before the birth of a human instead of a myeloid source [43]. Unlike hematopoietically derived Mφs, resident Mφs differentiated from erythro-myeloid progenitors are able to survive for a long time and self-renewal in specific tissues. With the lack of resident Mφs, it is generally believed that Mφs involved in bone regeneration are derived from cancelous bones in damaged or adjacent bones.

**The fate and polarization of Mφs**

The immune response mediated by the immune system is the host’s defense response to non-physiological stimulate factors, which is influenced by the expression level of inflammatory factors in the microenvironment [44]. As antigen-presenting cells in immune system, Mφs not only phagocytize pathogens or invaded foreign bodies, but also secrete cytokines to promote or inhibit inflammation [21, 22]. After being stimulated by chemokines, monocytes migrate from bone marrow to target regions through the circulatory system and differentiate into Mφs under the induction of macrophage colony-stimulating factor (M-CSF) and IL-4 [45]. In recent years, Mφs are considered to be necessary for tissue regeneration in vivo [46–48], which is contrary to previous studies. The absence of monocytes/Mφs inhibits osteoblast differentiation [21, 49], while the aggregation of monocytes/ Mφs induced by sphingosine-1-phosphate type I receptor agonist is able to significantly promote bone regeneration [50]. Before using Mφs in bone regeneration, a comprehensive understanding of the fate and polarization of Mφs is needed [51, 52].

The polarization of Mφs determines their different function in molecular biology. Under stimulation by different cytokines, Mφs in resting state (M0/Mφs) have the ability to polarize into classical activation phenotype (M1/Mφs) and alternative activation phenotype (M2/Mφs) [44, 53]. Moreover, some scholars further divided M2/Mφs into M2a/M2b/M2c subtypes [54]. Although both M1/Mφs and M2/Mφs originate from Mφs, they show significant differences in some aspects. Firstly, the cell morphology of M1/Mφs (pancake-like) and M2/Mφs (slender) is different [55]. Secondly, lipopolysaccharide (LPS) is necessary for Mφs to polarize into M1/Mφs under the regulation of STAT1 and NF-κB signaling pathways [56], while Mφs are more likely to polarize into M2/Mφs under the regulation of STAT6 signaling pathway when using IL-4 [55, 57]. The hardness of biomaterials is another potential factor to determine the polarization of Mφs. For example, hydrogels with high hardness induce Mφs to polarize into M1/Mφs, while Mφs in soft hydrogels are more likely to polarize into M2/Mφs [58]. Xie et al. have thought that these polarization differences are related to cytoskeleton recombination mediated by actomyosin and actin [59], and some other scholars have thought integrins and attachment molecules on the cell surface are more important for Mφs to identify biomaterials hardness [60, 61]. Finally, the cytokines secreted from M1/Mφs are not exactly the same. For example, current research results have shown that M1/Mφs mainly secrete tumor necrosis factor-α (TNF-α)/IL-1/IL-6/IL-12/IL-23/oncostatin M (OSM), accompanied by high expression of inducible nitric oxide synthase (iNOS)/CCR7/HLADR [57, 62, 63], while M2/Mφs mainly secrete IL-4/IL-10/IL-13/IL-1ra/vascular endothelial growth factor (VEGF)/insulin-like growth factor-1 (IGF-1), accompanied with high expression of CD163/CD206/Ym1/CCL1/CCL8/arginase-1 (Arg-1) [54, 64, 65].

The biological characteristics of M2/Mφs/M2/Mφs/M2/Mφs determine their roles in biological activities. He and his colleagues have found that M1/Mφs are able
to significantly promote the proliferation and adipogenic differentiation of BMSCs, while M₂Mφs have a stronger ability to promote the osteogenesis of BMSCs [53]. Although in their study, M₁Mφs only had a weaker promotion to BMSCs for their osteogenic differentiation than that of M₂Mφs, they have proved that M₂Mφs were more likely to induce BMSCs to form thick cell sheets. M₁Mφs mainly secrete pro-inflammatory factors to regulate the inflammatory response. Studies have shown that sepsis is caused by a high proportion of M₁Mφs with excessive expression of inflammatory cytokines (IL-6/TNF-α/IL-1) [66–68]. In contrast, the cytokines secreted by M₂Mφs are mostly anti-inflammatory factors which are beneficial to eliminate tissue inflammation and promote tissue regeneration.

The role of Mφs in bone healing

In recent years, the mechanism of Mφs in bone healing has been gradually explained, including secreting TNF-α/IL-1β to promote inflammatory response and secreting bone morphogenetic protein-2 (BMP-2)/OSM/stromal cell-derived factor-1 (SDF-1)/prostaglandin2 (PGE-2)/transforming growth factor-β (TGF-β) to promote tissue regeneration [21]. One of the potential theories to explain this mechanism is that exosomes derived from Mφs may have the ability to regulate bone homeostasis [78]. Mφs-derived exosomes release various cytokines and miRNAs to enhance cell communication in the microenvironment [79]. In the study of Li et al., exosomes derived from Mφs could inhibit inflammation and accelerate wound healing in diabetic animal models [80]. However, some studies have pointed out that the high expression of miRNA155 could also be found in exosomes secreted by M₁Mφs and it was thought to inhibit vascular regeneration and aggravate cardiac dysfunction in rat models [81]. miRNA222 is another kind of small molecule found in exosomes derived from M₁Mφs and it acts on the anti-apoptotic gene Bcl-2 to promote cell apoptosis (including BMSCs) [82].

Inflammatory factors secreted by M₁Mφs, such as TNF-α/IL-1/IL-6, mediate acute inflammatory responses after tissue injury [83]. Some scientists have thought that longer M₁Mφs infiltration is not conducive to tissue healing, due to the fact that it may strengthen tissue damage and influence the later regeneration processes [84–86]. However, it is worth mentioning that moderate inflammatory infiltration mediated by M₁Mφs is necessary and it is able to recruit stem cells into special regions to modify the microenvironment. In the study of Giannoudis et al., after adding nonsteroidal anti-inflammatory drugs, the process of bone healing was inhibited or even terminated [87]. TNF-α derived from M₁Mφs is able to enhance immune cells’ direct killing effect on damaged cells. But Glass et al. have found that low concentration of TNF-α would accelerate the process of bone regeneration by increasing the expression of RUNX2, OSX, ALP, and BMP2, which meant that the effect of TNF-α on the bone healing process was time-dependent and dose-dependent [88, 89]. Furthermore, VEGF secreted by M₁Mφs has the potential to promote angiogenesis and make regenerated tissues have sufficient blood supply [74]. Interestingly, lots of previous studies have shown that M₁Mφs only participate in the early osteogenesis stage and they are absent in the later bone mineralization stage [90, 91]. Compared to M₁Mφs, M₂Mφs have a stronger ability to promote tissue regeneration [8]. Anti-inflammatory factors secreted by M₂Mφs, such as IL-4, can enhance tissue vascularization and inhibit the activation of osteoclasts [63, 92, 93]. In addition to inhibiting tissue inflammation, IL-4 can also promote the transformation of M₁Mφs to M₂Mφs by activating the NF-κB signaling pathway and continuously increase the number of M₁Mφs in the microenvironment [94]. IL-10 and TGF-β are other cytokines secreted by M₂Mφs to inhibit the inflammatory response. Similar to M₁Mφs, M₂Mφs are able to induce the formation of primary vascular structures through secreting MMP-9 and promote anastomosis between neovascularization under the regulation of PDGF-BB [74]. M₂Mφs secrete more BMP-2 than M₁Mφs, which is conducive to the osteogenic differentiation of BMSCs [95]. BMP-2 enhances the nuclear transfer of RUNX2 by activating Smad1 signaling pathway and upregulates the expression of ALP and OCN in BMSCs [91, 96]. Furthermore, M₂Mφs at the fracture sites do not seem to be completely polarized from Mφs in bone marrow. For example, Doebel et al. have found that parts of M₂Mφs are migrated from the surrounding skeletal muscles in bone fracture models [97].
The existence of Mφs has a close relationship with endochondral ossification and granulation tissue formation in injured bones [21, 98, 99]. Compared with healthy individuals, mice without Mφs in bones show the characteristics of cartilage formation disorder and bone deposition obstruction. However, whether Mφs are necessary for cartilage formation is still controversial, due to the fact that in the embryonic stage, the cartilage primordium already exists before the development of Mφs lineage [100]. The ratio between M1Mφs and M2Mφs determines the balance between the process of inflammatory response and tissue regeneration in injured tissues [72, 101–103]. For example, the higher the proportion of M1Mφs in joint synovial fluid, the more severe the symptom of arthritis is [104]. Based on the previous research, the conversion time of M1Mφs to M2Mφs is usually 4–7 days [105]. Schlundt et al. have thought that if the transformation of Mφs was completed within 4–7 days, the later endochondral osteogenesis would not be affected anymore [99].

Although most studies have believed that Mφs are positive and necessary in bone healing, some scholars hold the opposite view. Tang et al. made Mφs and MSCs into 3D spheroids in a ratio of 1:1 for co-culture, and they found that the osteogenic ability of MSCs was significantly inhibited [106]. It may attribute to the negative role of N-cadherin in Mφs and MSCs. However, most current studies support M1Mφs and M2Mφs as well as their timely transformation is indispensable in bone regeneration.

**The regulatory effect of Mφs on the osteogenic differentiation of BMSCs**
Mφs not only promote bone healing, but also regulate the physiological functions of BMSCs through their paracrine effect. The extracellular matrix derived from Mφs maintains the integrity of microenvironment and protects cell–cell or cell–matrix communication in cell sheets [107, 108]. Mφs is important to the osteogenic differentiation of BMSCs and the crosstalk between these two kinds of cells is worth studying in the field of bone regeneration (Fig. 2).

Increasing shreds of evidence have suggested that Mφs affect bone healing by enhancing osteogenic differentiation and migration of BMSCs [21, 109, 110]. Jin et al. have prepared an intrafibrous mineralized collagen
(IMC) loaded with MΦs-derived exosomes and implant it into rat models. They found that this kind of biomaterial was able to significantly induce the osteogenic differentiation of endogenous MSCs through Smad1/5/9 signaling pathways [111]. Extracellular matrix proteins participate in cell–cell interaction through binding integrin adhesion receptors on the cell surface, which affect the migration, differentiation, and proliferation of stem cells [112]. In addition to Smad1/5/9 signaling pathways, several studies have proved that the STAT3 signaling pathway was also positive to the regulatory effect of MΦs on BMSCs [113]. OSM secreted by M1MΦs is considered to be necessary for the osteogenic differentiation of BMSCs, which is mediated by the OSM/NF-κB pathway [69, 71, 114]. Furthermore, Tu et al. have proved that IL-23 derived from M1MΦs also stimulates the osteogenic differentiation of BMSCs and it had relevance with the STAT3/β-catenin pathway [115]. In another experiment, when IL-23-p19 antibody was added to a co-culture medium, the osteogenic differentiation of BMSCs was significantly weakened [70], which further supported the Tu and his colleagues’ view.

M6A methyltransferase 3 (METTL3) has the ability to induce MΦs to polarize into M1 phenotype, which depends on the methylation of STAT1 mRNAs [116, 117]. M1MΦs are able to secrete TNF-α/IL-6/OSM/monocyte chemotactic protein-1 (MCP-1)/macrophage inflammatory protein-1α (MIP-1α) to recruit BMSCs into target regions [118, 119]. When METTL3 is selectively silenced in animal models, the secretion of these cytokines will significantly decrease and the decreasing formation of new bones will also be observed [120]. These results showed that the crosstalk between MΦs and BMSCs may be influenced by the methylation of some specific molecular structures in MΦs. Furthermore, some kinds of inorganic ions also play a positive role on the crosstalk between MΦs and BMSCs. For example, when studying the osteogenic induction effect of Mg^{2+} on MΦs, Qiao et al. have found an unknown mechanism about bone regeneration, including the increasing expression of CCL5/IL-8/IL-1ra and decreasing expression of IL-1β [121]. Although CCL5 and IL-8 are thought to be pro-inflammatory factors, they are able to recruit more BMSCs into damaged tissues to accelerate bone regeneration [122, 123]. Moreover, IL-8 is a key factor to promote revascularization and to keep the balance between bone reconstruction and resorption [124–126]. It should be pointed out that M1MΦs-derived exosomal miRNA5106 could induce the differentiation of BMSCs by targeting salt-induced kinases 2/3 [127]. Furthermore, miRNA378a containing in M1MΦs-derived exosome also promotes osteogenic differentiation of BMSCs by activating BMP signaling pathway [128].

Some studies have demonstrated that the crosstalk between MΦs and BMSCs has no positive effect on bone regeneration. Qi et al. cultured MΦs in hypoxia/serum deprivation conditions for obtaining the supernatant to inoculate with BMSCs and they found that the apoptosis rate of BMSCs in this specific medium was higher than that in the control group [129]. Then they added GM4869 into MΦs medium to inhibit the formation of exosomes and the new supernatant had no effect on the apoptosis of BMSCs. It is indicated that the apoptosis of BMSCs may be regulated by an unknown kind of substance in MΦs’ exosomes, and Qi et al. thought it should be miRNA222. In the study of He et al., both M1MΦs and M2MΦs conditioned medium inhibited the proliferation of BMSCs. They also found that the conditioned medium of M2MΦs had a stronger inhibitory effect than that of M1MΦs [53].

At present, the effect of MΦs on the osteogenic differentiation of BMSCs is still controversial, but most related research groups hold the view that M1MΦs enhance osteogenesis by recruiting more BMSCs to damaged sites in the early period, while M2MΦs focus more on bone matrix mineralization in the later period [95]. It requires more studies to verify MΦs’ effect on the osteogenic differentiation of BMSCs.

The function of BMSCs and their regulatory role on MΦs in bone healing
Stem cells have multi-directional differentiation potential to replace damaged cells in the cell pool and maintain tissue structural integrity. Since their discovery, stem cells have been often used in cell-based therapy for various diseases. At present, there are three main strategies for stem cells to be used in clinical treatment: (a) direct transplantation; (b) pre-differentiation before transplantation; (c) obtaining cytokines derived from stem cells [130, 131]. In order to explain the function of stem cells in various tissues and cellular crosstalk between them and microenvironment, Schofield and his colleagues created a well-established concept called stem cell niche.

The fate and biological characteristics of BMSCs
BMSCs have great potential in tissue regeneration engineering. In a previous study, Méndez-Ferrer and his colleagues used nestin to label MSCs to locate their distribution [26]. They found that although the majority of nestin+ MSCs located around blood vessels in the center of bone marrow, a small part of nestin+ MSCs could also be found near the bone intima. With low expression of HLA class I and II antigens, it is difficult for BMSCs to be captured by the host immune system [132], which significantly improve their survival rate in disease models, especially in allogeneic transplantation cases. Some
scientists think that the unlimited proliferation ability of stem cells makes it impossible to use them in the clinic due to their tumorigenicity (such as iPSCs and NSCs). However, there is no obvious evidence to show that BMSCs have this disadvantage [133, 134]. BMSCs have attracted great attention in the field of tissue engineering with their easy harvest, abundant quantity, and excellent cytological characteristics.

Recently, BMSCs have been proved to promote neural regeneration [135], skeletal muscle regeneration [136], vascular regeneration [137], and bone regeneration [138]. The differentiation of BMSCs is regulated by complex molecular mechanisms. Several classical genes are thought to be pivotal signals in the multi-differentiation of BMSCs. For example, RUNX2 regulates the osteogenic differentiation of BMSCs, PPARγ regulates the adipogenic differentiation of BMSCs, and Sox9 regulates the chondrogenic differentiation of BMSCs [139]. Once stimulated by the abnormal microenvironment, BMSCs will divide symmetrically or asymmetrically and migrate into injured tissues [140], which avoids excessive consumption of stem cells and ensures enough stem cells in specific regions. Furthermore, BMSCs also secrete kinds of cytokines to modify the microenvironment and eliminate inflammation, which is beneficial to accelerate the regeneration process. It is worth mentioning that any changes in the microenvironment will determine the fate of stem cells. For example, ASCs and BMSCs show obvious differences in differentiation potential, which strongly indicates that stem cells are deeply affected by their surrounding microenvironment. Recent research has demonstrated that cell–microenvironment communications are strong guarantees for stem cells to promote regeneration [107, 108].

The role of BMSCs in bone healing
BMSCs have been proved to be able to promote tissue regeneration in auto-transplantation cases [141–145]. Hernigou et al. injected bone marrow aspirate into injured bones and found obvious new bone formation [146]. It is valuable for scholars to explain how BMSCs migrate from the marrow cavity to damaged sites when a bone fracture occurs. Cells in injured bones release signal molecules to increase the expression of migration-related molecules (CD44) on the BMSCs surface. BMSCs can be fixed in appropriate areas through the connection between CXCR4 on cell surface and SDF-1 (high expression in injured tissues) derived from microenvironment [110]. MMPs with a high concentration in injured sites are able to dissolve extracellular matrix to produce sufficient space to accommodate migrated BMSCs and neovascularization [147, 148]. The chemotaxis of BMSCs would be enhanced in local hypoxia caused by a vascular rupture in the early bone injury stage, due to the fact that hypoxia could enhance the production of chemokines (SDF-1/CXCR12) [149, 150].

Bone regeneration, vascular regeneration, and neural regeneration are thought to be the main steps in bone healing. Ferrer uses BMSCs marked by fluorescence to prove their differentiation ability to reconstruct the Haversian system [26]. At present, scientists generally believe that RUNX2 is vital to the osteogenic differentiation of BMSCs and it can increase the expression of osteogenic-related proteins, including integrin bone sialoprotein (IBSP) and bone gamma-carboxyglutamate protein (BGLAP) [151]. Furthermore, BMSCs are able to differentiate into endothelial cells to reconstruct capillary networks in vitro. However, the capillary formation would rarely be observed in vitro if BMSCs are cultured alone unless specific cytokines are added, such as VEGF and epidermal growth factor (EGF) [152]. It seems that BMSCs are also involved in reinnervation in regenerative bone tissues, but some scholars do not support this view. Fu et al. have thought that only 8% BMSCs could be induced to form neurosphere-like structures in vitro [153], while Hermann have hold the view that this proportion could be risen to 60% by unique methods [154]. The different cytokines used by different researchers are the potential reasons for different results. However, the osteogenesis and angiogenesis of BMSCs should be the basis for BMSCs to reconstruct injured bones [155].

The paracrine of BMSCs is an effective mechanism to amplify their osteogenic-promoting effect [156–159]. The organic matrix secreted by BMSCs contains type I collagen fibers, BMP, IL-1, IL-6, and osteocalcin [160]. In the study of Gao et al., cytokines secreted from BMSCs promote the proliferation of endothelial cells and osteogenic differentiation of BMSCs in animal skull injury models [161]. Moreover, BMSCs upregulate the expression of VEGF to enhance vascular network formation, which is beneficial to increase oxygen supply in injured tissues [162]. However, some cytokines derived from BMSCs have been found to play a negative role in bone regeneration. For example, cytokine-like 1 (CYTL-1) was thought to inhibit the osteogenic differentiation of BMSCs by blocking RUNX2 and upregulating BAX protein expression in a recent study [163]. Shin et al. have proved that after being induced to osteogenic differentiation, BMSCs secrete less CYTL-1 than that in control groups in vitro, which meant that the regulatory effect of BMSCs on their osteogenic differentiation was bidirectional.

The regulatory effect of BMSCs on the immune response mediated by Mφs
Some scholars hold the view that BMSCs can be classified into BMSCs I phenotype (pro-inflammatory type)
and BMSCs II phenotype (anti-inflammatory type) under the regulation of Toll-like receptor [164]. They can be found in different bone remodeling stages, and their target cells include Mφs (Fig. 2). The classification of BMSCs is based on their immunomodulatory effect in bone healing. However, the academic community has not reached a consensus on this classification.

The crosstalk between BMSCs and immune cells is mediated by the connection among specific receptors on the cell surface [165, 166], which ensures that BMSCs are able to secrete anti-inflammatory factors (TGF-β and PGE2) to maintain bone immune homeostasis [21]. Furthermore, BMSCs are able to inhibit the proliferation of lymphocytes and induce the differentiation of regulatory T cells and dendritic cells. Due to their excellent anti-inflammatory properties, BMSCs have shown that excessive secretion of IL-4 could reduce transformation of Mφs. However, some research results have shown that M2Mφs to M1Mφs, and more IL-4, which forms a benign cycle to stimulate the transformation of Mφs [92]. IL-4 is sensitive to NF-κB signaling pathway and has the ability to further promote BMSCs to secrete more IL-4, which forms a benign cycle to stimulate the transformation of Mφs. However, some research results have shown that excessive secretion of IL-4 could reduce the osteogenic differentiation of BMSCs [94]. IL-1ra secreted by BMSCs is a natural inhibitor of IL-1, which blocks the connection between IL-1α and IL-1β or IL-1R through competitive binding to inhibit the polarization of Mφs [185] and the differentiation of dendritic cells [172].

Scientists believe that substances in exosomes derived from BMSCs activate several signaling pathways to inhibit tissue inflammation. Zhao and his colleagues have found that MSCs at the subpatellar fat pad secrete exosomes to accelerate the polarization of M1Mφs by increasing the expression of Arg-1 and IL-10 [173]. In the osteoarthritis animal models, BMSCs promoted the transformation of M1Mφs to M2Mφs, which inhibited cartilage degradation and enhanced cartilage synthesis [174]. The miRNA223 in BMSCs’ exosomes can stimulate pKNOX1 gene to induce Mφs to polarize into M2Mφs and accelerate wound healing [175]. In another study, Mao et al. have found that if miR-92a-3p in exosomes is overexpressed, the proliferation of chondrocytes would be enhanced with the decreasing expression of Wnt5α in tissue regeneration [176].

The regulatory effect of BMSCs on Mφs is complex, and it involves kinds of cytokines and signaling pathways. However, it seems that the function of BMSCs on Mφs only exists in the early stage of tissue regeneration. It is valuable for scientists to further explore how to make BMSCs continuously regulate inflammation and promote timely transformation of M1Mφs to M2Mφs in bone healing.

The prospects for the crosstalk between Mφs and BMSCs in bone healing

The ideal regeneration mode should positively regulate the hosts’ immune response to protect regenerative tissues and increase the number of stem cells [52, 177, 178]. Under the regulation of microenvironment, immune cells secrete chemokines to recruit stem cells into target regions and phagocyte pathological components in the early stage of tissue injury. The crosstalk between Mφs and BMSCs completes the transition from tissue inflammatory to regeneration under complex mechanisms in injured tissues. The regulatory effect of Mφs on BMSCs makes more stem cells migrate into target sites and secrete cytokines to induce Mφs to polarization toward suitable subtypes, which is positive to accelerate bone regeneration. In summary, stem cell recruitment, cytokine secretion, and signaling pathway activating are three core elements in bone healing [155].

However, in most cases, the crosstalk between Mφs and BMSCs in vivo is not enough to achieve the desired outcome of bone repairing due to cell deficiency, aging, and excessive tissue inflammatory. At present, the authors believe that how to enhance the crosstalk between Mφs and BMSCs by using biomaterials and regulate the inflammatory response in the aged population will be the notable research field in the future.

The regulatory effect of biomaterials on Mφs and BMSCs

The effect of biomaterials on osteogenesis mainly depends on their biocompatibility. Biomaterials were
thought to be recognized by immune system and subjected to immune attack in vivo due to their heterogeneity in the past. Foreign body reaction (FBR) and fiber wrapping often lead to unsuccessful biomaterial implantation [179, 180]. Therefore, biomaterial implantation has exact potential negative feedback on inflammation elimination and tissue regeneration. However, the modification of biomaterials has solved the above problems and expanded their application in stem cell-based therapy. Biomaterials can be used as cell carriers to provide shelters for transplanted cells to avoid excessive infiltration, which ensures the survival of exogenous cells [181]. Furthermore, some biomaterials can slowly release anti-inflammatory factors or growth factors loaded on them in vivo and it regulates inflammation in the microenvironment to facilitate tissue regeneration. For example, in order to maintain a suitable concentration of Mg$^{2+}$ locally, Qiao et al. have synthesized a kind of hydrogel which would continuously release Mg$^{2+}$ for 7 days after implanting [121]. Perfect biomaterials must be able to stimulate moderate inflammatory response, release cytokines to nourish stem cells, and integrate new tissues (Fig. 3) [182].

The biocompatibility of biomaterials can be determined by their effects on the balance between M1 type Mφs and M2 type Mφs in injured tissues. Chen and his colleagues added tricalcium phosphate (TCP) particles into Mφs medium and they observed the increasing number of M2 type Mφs and high expression of BMP-2 in medium [183, 184]. In another study, scientists transplanted TCP particles loaded with histone methyltransferase enhancer of zeste1 (EZH1) into animal models and they found that the proportion of M2 type Mφs was significantly increased compared with that of in the control group [44]. Moreover, another study recently has shown that β-TCP particles had the ability to enhance the osteogenic differentiation of BMSCs by inducing the polarization of Mφs and regulating the Wnt signaling pathway [185]. However, the function of TCP particles on bone immune system is still in dispute. Some researchers added cobalt (Co) into TCP particles and found that Co-TCP composites improved the proportion of M1 type Mφs and enhanced bone absorption [186]. The reason for different results in studies may be that the cytokines loaded with TCP particles were not the same. However, as natural components in bones, TCP particles are necessary for the physiological function of bones. In the study of Liu et al., IMC had the ability to promote Mφs to secrete functional exosomes to accelerate bone regeneration under the regulation of the BMP2/Smad5 signaling pathway [111]. Interestingly, when Mφs co-culture with graphene oxide (Go), the inflammatory response will be strengthened and the osteogenic differentiation of BMSCs will also be enhanced by activating OSM/NF-κB signaling pathway [69, 71]. Ujiie has designed a kind of scaffold materials with releasing interferon-γ (IFN-γ)/IL-4 in sequence. They also proved that it was positive for the polarization of Mφs and revascularization in regenerative tissues [187].

The regulatory effect of biomaterials on BMSCs also profoundly affects the fate of Mφs. Gamblin et al.
implanted BMSCs on biphasic calcium phosphate (BCP) particles to construct a new BMSCs-biomaterial system and observed obvious chemotaxis of Mφs [188, 189]. Xue et al. have found that after inoculating BMSCs in Cu-MSN/Mφs conditioned medium, the osteoprotegerin (OPG) secreted by BMSCs was significantly upregulated accompanied by the downregulation of RANKL, both of which were positive for bone regeneration [69]. In other several studies, scholars transplanted fibrin and hydroxyapatite as cell carriers into experimental animals and found that the majority of Mφs polarized into M1Mφs in the early stage, but it is interesting that M1Mφs would be transformed into M2Mφs in the later stage. The reason for it may be that exogenous BMSCs added groove structures [176].

The aging effects on Mφs and BMSCs
The typical characteristic of aging is the functional degradation of tissues and cells. Aging has obvious effects on all kinds of cells, especially adult stem cells and Mφs. Mahbub et al. have found that aged M2Mφs secreted less IL-1β and TNF-α than younger ones, which caused a mild inflammatory reaction when tissues were injured [200]. In contrast, another study conducted by Brett has demonstrated that aged M1Mφs mediated more severe inflammatory response and prolonged inflammation process [201]. The latter view seems to be supported by some research that focused on this topic. For example, Gibon and his colleagues have proved that aged M1Mφs significantly upregulated the expression of pro-inflammatory factor (TNF-α) and down-regulated the expression of anti-inflammatory factor (IL-1ra). It also explained why tissues in the elderly were always in a high inflammatory state [201, 202]. Furthermore, after interconnecting the circulatory system of aged rats with young rats, inflammatory response inhibition and bone regeneration acceleration could be clearly seen in aged groups [203].

BMSCs were more sensitive to aging than Mφs. With age increasing, the weak biological function and apoptosis of stem cells slow down the tissue regeneration process. Fat tissues will gradually accumulate in the bone marrow cavity to affect the osteogenic differentiation ability of BMSCs in aged groups [204]. In contrast, the adipogenic capacity of BMSCs will gradually enhance with aging, which also weakens the process of bone regeneration [205]. The increasing adipogenic capacity of BMSCs with aging may be attributed to the decreased expression of transcriptional coactivators containing PDZ binding sequences, which leads to the increasing expression of PPARγ and decreasing expression of RUNX2 [206]. The low metabolic capacity and strong adipogenic capacity of BMSCs in elderly individuals lead to a large accumulation of adipose tissues in bone marrow, which brings a fatal blow to the self-healing in patients with fracture.

Aging makes old patients not only suffer from more pain during fracture healing, but also get the worse healing outcome than young patients. How to solve this problem is another aim for scientists in the field of tissue engineering.

Conclusion
In recent years, studies on crosstalk between Mφs and BMSCs have suggested that the bone healing process is complex. Inflammatory mediated by Mφs plays a vital role in bone regeneration. On the one hand, excessive inflammatory responses may cause irreversible tissue damage and affect adjacent healthy tissues. On the other hand, mild inflammatory responses activate signaling pathways that are related to tissue regeneration to repair damaged tissues. The inflammatory response degree depends on the proportion and transformation of
M1-Mφs and M2-Mφs in the microenvironment. BMSCs are another important kind of cells in bone healing. Both the osteogenic differentiation of BMSCs and cytokines derived from BMSCs are necessary to bone regeneration. The effect of crosstalk between Mφs and BMSCs is significant to the outcome of bone healing. The inflammatory mediated by Mφs is able to regulate the osteogenic differentiation of BMSCs, and meanwhile, the cytokines secreted by BMSCs are also able to inhibit or stimulate the inflammatory response in bones. Therefore, more studies are needed to focus on how to regulate the crosstalk between BMSCs and Mφs to accelerate bone healing. The recent research on biomaterials provides us with new methods to solve problems about the insufficient number of stem cells and excessive inflammatory responses. The stem cell–biomaterial models are not only able to carry seed cells, but also to load with various necessary cytokines, which significantly modify the microenvironment in damaged tissues. The emergence of biomaterials can also effectively improve prognosis due to high inflammatory status of tissues in elder patients. The development of new biomaterials and modification of existing biomaterials are effective methods to improve tissue regeneration, and it demands more research for further exploration and discussion.

Abbreviations
Arg-1: Arginase-1; ASCs: Adipose stem cells; BCP: Biphasic calcium phosphate; BGLAP: Bone gamma-carboxyglutamate protein; BMP-2: Bone morphogenetic protein-2; BMSCs: Bone marrow mesenchymal stem cells; Co: Cobalt; CYTL-1: Cytokine-like 1; EGF: Epidermal growth factor; EZH1: Histone methyltransferase enhancer of zeste 1; FBR: Foreign body reaction; Go: Graphene oxide; HLA-DR: Human leukocyte antigen-DR; IBSP: Integrin bone sialoprotein; IFN-γ: Interferon-γ; IGF-1: Insulin-like growth factor-1; IMC: Intrafibrous matrix; ITPA: Interphalangeal type A; MCP-1: Monocyte chemotactic protein-1; M-CSF: Macrophage colony-stimulating factor; Mε: Melanocyte; MTR: Methionine transferase; Mφ: Macrophage; MIR: MicroRNA; MMP-9: Matrix metalloproteinase-9; NPT: Nuclear protein tRNAase; OPG: Osteoprotegerin; OSM: Oncostatin M; PGE2: Prostaglandin E2; PDGF-BB: Platelet-derived growth factor-BB; PGE2: Prostaglandin E2; SDF-1: Stromal cell-derived factor-1; TGF-B: Transforming growth factor-β; TNF-α: Tumor necrosis factor-α; VEGF: Vascular endothelial growth factor.

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Author contributions
YHW contributed to the design of the conception, drew pictures, reviewed the literature, drafted and critically revised the manuscript. CZZ, RYW, and XQD reviewed the literature, revised the manuscript, and designed the conception. JYL and JP designed the conception and critically revised the manuscript. All authors gave the final approval and agreed to be accountable for all aspects of the work. All authors read and approved the final manuscript.

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