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Interactions between multipotential stromal cells (MSCs) and immune cells during bone healing

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Abstract:

The physiological process of bone healing takes place in three sequential stages: inflammation, repair and remodelling. Multipotential stromal cells (MSCs) are the key progenitor cells for osteoblasts and chondrocytes and are also imbued with immunomodulatory capabilities. Although MSCs are well known to be involved in osteogenesis during the later stages of repair, their role during the inflammatory phase and precise interactions with immune cells remain poorly understood. This chapter describes the current knowledge on cellular interactions during the bone repair as well as cytokines and growth factors mediating these processes. The roles of emerging innate immune cell populations, innate lymphoid cells are also discussed. Based on this current knowledge, we conclude that in addition to their differentiation during later bone repair stages, MSCs are likely to have a substantial involvement in the initial stage of bone healing by controlling the fate of inflammation. An improved understanding of complex cell interactions during bone repair has broad implications on optimising the treatment of the fracture complications including non-union.

Key words:

Bone repair, MSCs, immune cells, bone fracture.

I. Introduction

Human bone has a unique structure formed of outer dense layer of cortical bone responsible for the weight bearing function. The outer bone is covered by the periosteum that has a contributing role in the osteogenesis and the removal of the periosteum delays significantly the bone repair (1). The inner part of the bone is a mesh-like cancellous bone containing bone marrow (BM) where various cell types including multipotential stromal cells (MSCs) exist (2). The first description of MSCs was as colony-forming unit fibroblasts with capability to generate a new bone in ectopic sites (3). Later on, they were renamed as mesenchymal stem cells (4). According to the International Society of Cell Therapy (ISCT), MSCs are defined in vitro as plastic adherent cells, which do not express hematopoietic lineage markers CD45, CD34, CD14 or CD11b, CD79 alpha, CD19 and HLA-DR, but they express surface molecules CD90, CD73, CD105. Thirdly, MSCs are able to differentiate into osteoblasts, adipoblasts and chondroblasts (5).

There are many tissues where MSCs can exist in skeleton including the periosteum, bone, BM, muscles, tendons, ligaments, and adipose tissue (6). In animal models, MSCs can migrate from BM into blood then circulate and home to the injury site where they can be active in repairing tissues (7). The most common and best-characterised source of MSCs is BM. The surface molecule CD271 is currently considered as a distinctive and selective marker for human BM and bone MSCs (8, 9). Interestingly, the periosteum contains MSCs with chondrogenic and osteogenic potential that is comparable to BM MSCs (10). Furthermore, periosteal MSCs are higher in quantity relative to BM MSCs in canine models (11) but it is unknown if periosteal MSCs are similarly abundant in human.

The proliferation of MSCs is closely linked to their differentiation function. Rapidly growing MSCs have superior differentiation abilities compared to slower proliferating MSCs (12). In addition to mesenchymal tissue differentiation, MSCs can display immunomodulatory functions, mostly suppression of the proliferation and functions of different immune cells (13). Critically, MSCs need to be primed by inflammatory cytokines to play their immunomodulatory role (14). With the advancement of the examination of the surface phenotype and the functional characterisation of MSCs from multiple tissue sources, the importance of MSCs as key cells in bone healing becomes better understood.

Although the bone healing is a successive process, its phases are overlapping to some-extent, inflammation, repair phase including chondrogenesis followed by ossification then finally remodelling phase. When a fracture or bone loss occurs, the injury-driven rupture of

blood vessels and vasodilation causes an exudation of plasma into the surrounding tissues. Plasma-derived fibrinogen is converted into fibrin forming a haematoma which traps various inflammatory cells and MSCs (15). The function of inflammation is the clearance of the damaged tissue and the initiation of the repair by providing the needed cells and the appropriate cytokines and growth factors (16). Subsequent to the inflammatory phase, a repair phase starts with differentiation of MSCs into chondroblasts, which proliferate and differentiate, forming a cartilaginous thickened structure called soft callus. Then chondroblasts undergo hypertrophy and begin to deposit mineralised matrix and soft callus is converted into new bone (hard) callus. This process is known as endochondral ossification leading to the formation of irregularly arranged (woven) bone. During this phase new blood vessels are formed in a process called angiogenesis. The final phase includes remodelling of woven bone into lamellar bone i.e. normally aligned bone (17-20).

Intramembranous repair involves a direct differentiation of MSCs into osteoblasts and it usually happens when broken bone edges are perfectly aligned (direct or primary healing) (17). In addition to MSCs, bone cells including osteoclasts and osteoblasts also play important roles in bone healing. Osteoclasts are giant multinucleated cells originated from monocyte lineage cells and express tartrate-resistant acid phosphatase. The main function of osteoclasts is bone resorption through release of the enzymes and other molecules, which degrade the bone matrix (21). Osteoblasts are bone-forming cells originated from MSC-derived progenitors. The balance between the functions of these bone cells and immune cells, helps to maintain the normal quantity and the function of the regenerating bone and is critical to determine the fate of the healing process (22). In this chapter, the role of various immune cells, cytokines, bone cells and MSCs and their interactions during different phases of the bone healing in health and as well as in exaggerated inflammatory milieu are reviewed (Figure 1, 2 and 3, Table 1 and 2).

II. Inflammatory phase

Inflammation taking place at the fracture site is caused by the damage of tissues, hypoxic and low pH environment. Inflammatory phase involves several events including recruitment of different blood cells and release of various inflammatory chemokines and cytokines (16, 23). Immune response is critical for physiological uncomplicated healing of fractured bone. The transfer of immune cells from young mice via BM transplantation into immune-compromised mice improves significantly the process of bone repair (24). Furthermore, immune-compromised patients such as HIV-infected individuals show delayed or complicated healing

of bone fractures (25). Although different factors could be involved in HIV-related fragility of healing bones, immune-related mechanisms have been demonstrated. For example, serum level of both Tumour necrosis factor-α (TNF-α) and the TNF receptors are increased in these patients in comparison to healthy controls (26). Cells and molecules of innate and adaptive immune systems as well as MSCs are involved in inflammatory phase of bone healing to maintain tissue haemostasis through removal of the damaged cells and induction of cartilage and bone repair in addition to angiogenesis (27). The role of different immune cells and MSCs as well as other inflammatory soluble factors are discussed below.

i. Neutrophils

The first cells to be recruited into hematoma at the injury site are neutrophils. In a rat model of tibia fracture, neutrophils were detected at the fracture callus on day 1 and remained for few days after the fracture (28). In association with high-level of chemokine, cytokine-induced neutrophil chemoattractant-1 (CINC-1), neutrophils migrate across the endothelium into hematoma site (29). Neutrophils are important to eliminate the microbial infection (30). The functions of neutrophils at the fracture site also include phagocytosis of the damaged cells and debris (27). Although they have short life span, neutrophils play antiseptic role at the fracture site, but it remains unknown if they interact with MSCs.

ii. Macrophages and monocytes

Macrophages are recruited into the fracture site from the periosteum or peripheral blood (31, 32). Macrophage-colony stimulating factor (M-CSF), which is known to target mainly macrophages (33), is expressed in the fractured bone, peaking during inflammatory phase then again when remodelling phase starts (34) indicating an involvement of the macrophages during all phases of the repair. Macrophages and platelets produce bone morphogenetic proteins (BMPs), which contribute in organising the shape of the different tissues particularly bone and cartilage (35). BMPs are known to stimulate the proliferation of MSCs and enhance their osteogenic differentiation (36). Another molecule, Oncostatin M is produced by monocytes/macrophages and induces osteogenic markers, Core-binding factor alpha 1/runt-related transcription factor 2 (Cbfa1/Runx2) and alkaline phosphatase (ALP) in MSCs enhancing their differentiation into osteoblasts (37, 38). Additionally, monocyte-derived exosomes also increase the bone-forming function of MSCs via the induction of the expression of Cbfa1/Runx2 and BMP2 in MSCs (39). Monocytes activated by interleukin-4 (IL-4) or lipopolysaccharide (LPS) elevates the expression level of Cbfa1/Runx2 and ALP by MSCs

(40). Thus interactions between MSCs and macrophages induce the osteogenic ability of MSCs preparing for the repair phase.

iii. NK cells

Natural Killer (NK) cells constitute an important part of innate immune system and are divided into two functional subsets; bright CD56 (cytokine producers or regulatory NK cells) and dim CD56 (killers). NK cells can circulate and reach different organs where they become activated and function to keep immune haemostasis in different tissues (41). Up to date, little is known about the biological roles of NK cells during the bone healing. One study has described a high expression of interferon-γ (IFN-γ) in the tissues of diaphyseal regions of fractured femur in $RAG^{-/-}$ mice model, which lack both T and B lymphocytes (42). As a major source of IFN-γ, this study has pointed to the importance of NK cells during bone repair. This role is assumed because NK cells produce a major priming cytokine for immunomodulatory function of MSCs, IFN-γ (42). Yet, further research is needed to understand the magnitude of NK cell-dependent priming of MSCs. The inflammatory milieu as well as MSC-NK cell interactions could modify activated NK cell functions to increase the production of IFN-γ (43). NK cells are also major source of TNF-α, which has a critical role in bone repair. Mice lacking TNF-α have been shown to have defective bone generation (44, 45). In addition to priming effects, NK cells have been shown to enhance the migration of MSCs through matrigel inserts (46).

NK cells stimulate the generation of osteoclasts through release of Receptor activator of nuclear factor kappa-B ligand (RANKL) (47). Data from in vitro studies has shown that RANKL and TNF-α also trigger monocyte differentiation into osteoclasts (48). This suggests that NK cells can indirectly, via effect on osteoclasts, help the removal of debris and damaged tissues in physiological bone repair. In summary, NK cells can induce MSC functions including the priming and the migration via their cytokines in addition to their effect on osteoclasts all indicate their important contribution into the bone healing.

iv. Innate lymphoid cells

Innate lymphoid cells (ILCs) are newly characterised immune cells originated from common lymphoid progenitor but they lacking lineage markers for T or B lymphocytes (49, 50). ILCs are divided into three functionally diverse subclasses. NK cells are considered as a subtype of ILC1. ILCs have common receptors for IL-12 and IL-7 but have heterogonous functions with distinctive pattern of cytokine profiles (51, 52). While ILCs 1 secret Th1 cell-like cytokines, TNF-α and IFN-γ (53, 54), ILCs 2 produce IL-4, IL-5, IL-9 and IL-13 similar to Th2 cells (55-

57). In contrast, ILCs 3 have similar profile to Th17 as they produce IL-17 in addition to IL-22 and M-CSF (58). Interestingly, ILC3 help the regeneration of damaged inflamed intestine, thymic and lymphoid tissues via their production of IL-22 (59-61). Furthermore, ILCs 2 play important role in repair against viral-related damaged of bronchial epithelium (62).

Compared to their role in epithelial and lymphoid tissues, little is known about the role of these innate cells during the bone healing process. Interestingly, one study showed that IL-17 putatively produced by ILCs 3 could induce the expression of Cbfa1/Runx2 as well as late osteogenic marker, collagen in MSCs (63). Although this group indicated that Th17 cells could induce osteogenesis, a similar effect of ILCs 3 can be proposed, as they are another source of IL-17 (63). Furthermore, it has been shown that following priming with IFN-γ and TNF-α, IL-22 stimulates the migration and the proliferation as well as osteogenic differentiation of MSCs in vitro (64). This all suggest that these innate immune cells probably work during later stage of inflammatory phase to prepare MSCs for next phase of the bone repair. However, there is a need to locate and study the functions of these ILCs within healing bone tissues.

v. T and B lymphocytes

During inflammatory phase, the acquired immune cells, T and B lymphocytes are important in bone healing. In animal models where T and B are depleted, a defect in bone mineralisation and delayed healing has been described (65-67). T and B lymphocytes are recruited at the bone fracture site after 3 days of injury and then reduced in numbers with the start of cartilaginous callus formation as shown in animal models (63, 68). RANKL that is made in part by activated T lymphocytes in addition to stromal cells and NK cells stimulates osteoclasts to remove the dead bone (69, 70).

Importantly, T lymphocytes are linked to the priming of immunomodulatory function of MSCs as T lymphocytes secret TNF-α, the cytokine that peaks during both inflammatory and repair phases (34). Additionally, conditioned media from the cultured CD4 T lymphocytes but not CD8 T lymphocytes can induce the expression level of both early and late osteogenic markers, Cbfa1/Runx2, ALP, osteocalcin and Bone sialoprotein (BSP) by MSCs (71). Th17 cells are positive inducers of the osteogenic potential of MSCs and IL-17 enhance osteogenic markers in MSCs both in vitro and in vivo (63). Similarly, T regulatory lymphocytes (T reg lymphocytes) are also assumed to be involved in bone healing, as they are major source of TGF-β, which promote MSC proliferation and osteogenesis (72). Members of TGF-β family, TGF-β2 and TGF-β3 showed maximal expression when inflammation ends and cartilage formation starts, as they play their role as immunosuppressive cytokines thus controlling the

inflammation (73). Altogether, this illustrates how T lymphocytes can enhance the osteogenic potential of MSCs in preparation to the next phase of repair.

There is some conflicting data about the role of B lymphocytes in bone repair. While it has been shown that B lymphocytes enhance osteoclast formation (74); another study showed that B lymphocytes suppressed osteoclastogenesis and promoted death of osteoclasts via TGF-β expression (75). Furthermore, in B lymphocyte deficient mice model of tibial injury, B cells did not affect osteoblast differentiation or maturation during intramembranous ossification (76). Remaining inconclusive, there is a need for more studies to understand if B lymphocytes have other roles during the bone repair via interactions with MSCs.

vi. Multipotential stromal cells

Source and Recruitment

Hematoma formed soon after the bone fracture consists of granulation tissue holding various cells including MSCs (15, 77). The origin of these MSCs could be the periosteum, BM or vascular endothelium-supporting cells, pericytes (78-80). Periosteal-derived MSCs have been shown to form bone and cartilage with a help of BMP-2 for their differentiation (81). In cases of open fractures where the periosteum is stripped, MSCs can be also derived from adjacent muscle tissue (82). MSCs are recruited into the fracture site under the effect of an inflammatory chemokine, Stromal Derived Factor-1 (SDF-1) as revealed in a mouse model of femoral injury (83). By the activation of alpha serine/threonine-protein kinase (AKT) and extracellular-signal-regulated kinases (ERK) signalling pathways, SDF-1 induces the migration of MSCs and the secretion of angiogenic factor, Vascular endothelial growth factor (VEGF) (84) (85). SDF-1 is also an important inducer for BMP-2, which activates MSCs and promotes osteogenesis (86). Another cytokine, which mediates the invasion of MSCs into the injury site, is TNF-α that acts via NF-κβ-dependent signalling pathway (87). The multiplicity of sources of MSCs reflects the vitality of these cells in the bone repair.

The early role of MSCs during inflammatory phase

Although MSCs have been detected in fracture hematoma, their role in early phase of fracture repair is not completely clear. One in vitro study has suggested that MSCs could be involved in phagocytosis of dead cells at the fracture site. This process is associated with an increase of the expression of chemokine receptors, CXCR4 and CXCR5 by MSCs helping recruiting more MSCs into the injury site (88). The exposure of T lymphocytes to these phagocytic MSCs could increase the expression of RANTES thus recruiting more CD4 T lymphocytes to the

injury site. Furthermore, these MSCs could stimulate the differentiation of Th-17 cells that are linked to osteogenesis (88). Another study has indicated that MSCs could induce the formation of osteoclasts with an involvement of IL-6 and M-CSF (89). In conclusion, MSCs seem to help removal of bone debris directly or by enhancing osteoclastogenesis. Additionally, MSCs could induce further recruitment of MSCs and T lymphocytes into the fracture site.

Priming of MSCs during early inflammatory stage

Inflammation-mediated priming of MSCs is critical to complete the bone healing. IFN-γ can activate the proliferation and immunosuppressive function of MSCs via the activation of the Kynurenine pathway (90). Concomitant with IFN-γ, TNF-α or IL-1 increase the expression of adhesion molecules, ICAM-1 and VCAM-1 on MSC surface helping their contact with T lymphocytes and causing an augmented MSC-mediated inhibition of T cell proliferation (91). It has been shown that TNF-α also helps the proliferation of MSCs and the initial priming of immunosuppressive function of MSCs via NF-κβ-dependent mechanism (92). The inflammatory cytokine, IL-1 mediates its effect on MSCs via multiple signalling pathways including map kinase (MAK), Jun amino-terminal kinase (JNK) and NFκ-β. Furthermore, the downstream activation mediated by IL-1 controls the production of immunomodulatory factors, prostaglandin 2 (PGE2) and IL-8 by MSCs (93).

Within inflammatory milieu, the activation of toll-like receptors (TLRs) on the surface of MSCs induces MSC migration, differentiation, proliferation and production of indoleamine 2, 3-dioxygenase (IDO) and PGE2. The stimulation of TLR activates MAP kinase, NFκ-β and PI3 kinase signalling pathways (94). Recently, IL-17 was added to the list of priming factors that induce MSC immunomodulatory effect. IL-17 can induce the production of inducible nitric oxide synthase (iNOS) by MSCs as tested in vitro and in vivo (95).

In contrast to these priming cytokines, TGF-β can act as a counterbalancing cytokine to decrease the immunosuppressive effect of MSCs. Uniquely, TGF-β affects both immunomodulatory and repair functions of MSCs (96). One mechanism by which TGF-β affects MSCs immunomodulatory function is the decrease of iNOS expression in SMAD3-dependent manner (97). Similarly, immunosuppressive cytokine, IL-10 abrogates the inhibitory effect of MSCs on the proliferation of CD4 and CD8 T lymphocytes (98). However the role of these counteracting cytokines during the inflammatory phase of bone repair is probably less evident compared to MSC priming cytokines. The expression levels of TGF-β 2 and 3 are increased during cartilaginous callus formation to help differentiation of MSCs (73). In conclusion, MSC priming and counteracting cytokines are likely to work synergistically to

prime MSCs for their immunomodulatory effects towards the transition to the repair stage of bone healing.

The late role of MSCs during inflammatory phase; immunomodulation

The regulation of immune response within the fracture environment is critical to reduce the tissue damage, inhibit fibrosis supporting the bone regeneration (27, 34). This important role of MSCs controlling inflammatory phase helps the transition into the next stage of bone healing process (99). The discovery of an ability of MSCs to regulate immune systems was made in late 1990s/early 2000s by the observation that MSCs suppress the proliferation of T lymphocytes in vitro and in vivo, indicating an additional function of MSCs (100, 101). The subsequent studies have proven that only inflammatory-primed MSCs can mediate immunomodulation of both innate and adaptive immune responses. The effect of MSCs on IL-12 or IL-15 activated NK cells includes the suppression of their proliferation, killing and secretory functions (102, 103). The differentiation of T lymphocytes into Th1 and Th17 subsets can be suppressed by MSCs, however MSCs promote the differentiation of T reg lymphocytes (104). Additionally, MSCs can induce dendritic cells to trigger T reg generation via the production of IL-10 by these dendritic cells (105). Furthermore, MSCs induce the apoptosis of T lymphocytes that trigger macrophages to secrete TGF-β inducing the expression of T reg lymphocytes (106). Moreover, MSCs have the ability to suppress the function and the migration of B lymphocytes via the down-regulation of the chemokine receptors expression; CXCR4, CXCR5 and CCR7 on the surface of B lymphocytes (107). Overall, this indicates that MSCs interact with both innate and adaptive immune cells controlling whole inflammatory response.

The mechanisms by which MSCs exert their immunomodulatory effects are mainly related to soluble factors. The soluble immunomodulatory molecules include TGF-β, IDO, iNOS, PGE2, IL-1 receptor antagonist and Tumor necrosis factor-inducible gene 6 (TSG6) (108-111). The use of these different players by MSCs varies according to host species, tissue type or priming microenvironment of MSCs (112). For example, mice MSCs mainly use nitric oxide (NO) to modulate immune responses, but IDO is the main player for human MSCs (112). In summary, MSCs exert a negative feedback loop to control the inflammation at the fracture site.

The summary of inflammatory phase

Under the normal condition, inflammatory phase is important initial phase of the bone healing (Figure 3). Neutrophils are the first cells to arrive at the fracture site preventing sepsis. NK cells, TNF-α in addition to SDF-1 help to recruit MSCs. MSCs directly and via the activation of osteoclasts help the removal of the dead cells and debris. Other immune cells, B and T lymphocytes also stimulate the formation of osteoclasts. Inflammatory cytokines and growth factors enhance the proliferation of MSCs as well as priming of MSCs to exert their immunomodulatory effects. As a negative feedback mechanism, primed MSCs then are able to suppress the inflammatory responses and help to start the repair phase of bone healing. Nevertheless, the effects of some inflammatory cells continue to play their roles during the repair phase (Figure 1 and 2).

III. Repair phase

The repair phase includes the formation of cartilaginous callus in the secondary bone healing (endochondral ossification) from the enlarging cartilaginous tissue patches filling the bone defect site (113). On the other hand, the primary bone healing involves the formation of hard or bone callus that happens in the absence of cartilaginous callus (intramembranous ossification) (114). The endochondral ossification is the most common healing type and includes the degradation of cartilaginous callus, which then is replaced by the bone callus in association with the formation of new blood vasculature. The role of MSCs and immune cells as well as soluble mediators is discussed below.

i. MSCs

During the repair phase, MSCs proliferate and differentiate into chondroblasts that mature into chondrocytes, which deposit cartilaginous matrix (115). Chondrocytes mature and deposit calcium granules into matrix where they precipitate with phosphatase forming apatite crystals under hypoxic conditions. During these events and while a mechanically rigid bone callus is forming, a high expression of osteogenic markers, procollagen-I, osteocalcin, ALP and osteonectin becomes evident (17, 116).

The second role for MSCs during this stage includes the production of VEGF under control of Cbfa1/ Runx2 transcription factor (117). BM MSCs express angiogenic factors VEGF and angiopoietin-1 (118). Thus, MSCs are considered the key cells initiating this phase by chondrogenic differentiation as well as induction of angiogenesis.

ii. Macrophages

Macrophages are known to be involved in the intramembranous ossification during the repair phase (31, 32). Macrophages also participate in the bone repair via the induction of angiogenesis. A significant decrease in macrophages in $CCR2^{-/-}$ mice was associated with impaired vascularisation and delayed formation of callus (31). Macrophages are present in invading vessels during the ossification of mouse long bones (119).

Later during this phase, macrophages can efficiently secrete matrix metalloproteinases (MMPs) to break the cartilage matrix (120, 121). MMP-9 and MMP-13 have important contribution during soft-to-hard callus conversion (122-124). Matrix metalloproteinases and their inhibitors help the conversion of collagen II to type I; and the disturbances in the regulation of these enzymes are connected to the fracture non-union (125). In a mouse model of tibia fracture, both resident macrophages and inflammatory circulating macrophages were shown to be vital for the deposition of collagen type I (126). Furthermore, it has been shown that the gene expression of macrophage macrosialin protein is positively associated with the expression of collagen in fractures (127). In summary, macrophages are active during whole repair phase working synergistically with MSCs to help forming of collagen matrix and formation of new blood vessels.

iii. T and B lymphocytes

Although they disappear at the end of inflammatory phase and during formation of callus, T and B lymphocytes reappear during the mineralisation of cartilaginous callus and have been detected in a close contact with osteoblasts and osteoclasts (128). Both types of adaptive immune cells are recruited into newly forming hard callus presumably via new blood vessels to produce more TNF-α, the cytokine responsible for the death of mature chondrocytes. Additionally, TNF-α stimulates the maturation of osteoblasts and osteoclasts helping the conversion from cartilage into bone (44, 45). Overall, T and B lymphocytes have a role in the late stage of bone healing helping the transition of cartilaginous callus into bone callus. This role seems to be mediated via TNF-α to regulate the function of osteoblasts and osteoclasts.

iv. Growth factors and cytokines

Different growth factors including, platelet-derived growth factor (PDGF), TGF-β, Insulin-like growth Factor (IGF) and fibroblast growth factor-1 (FGF-1) promote the proliferation and the differentiation of MSCs into chondrocytes (129) (114). Similarly, BMPs-2, 4 and 7 induce the differentiation and proliferation of MSCs (130). Furthermore, BMPs stimulate the

chondrocytes to secret extracellular matrix proteins such as collagen type II (36). The importance of these factors has been established and it has been shown that the gene expression levels of BMPs is significantly lower in the sheep model of delayed bone union compared to controls (131). Furthermore, the depletion of BMPs causes severe defects in the bone formation as seen in animal models, whereas the treatment with recombinant BMPs improves the fracture repair (132-134). This indicates that multiple growth factors help chondrogenesis at the start of the repair phase.

The bone fracture needs a good vasculature. Angiopoitein-1 and -2 proteins are expressed early promoting the vascularisation from periosteal capillaries (135). VEGF is another key factor in angiogenesis and it is produced in a large quantity from MSCs and differentiated chondrocytes converting avascular soft callus into vascularised bone callus. For this process to be completed, the death of chondrocytes and the breakdown of cartilaginous callus are happened in TNF-α-dependent mechanism (136). Importantly, TNF-α signalling in chondrocytes increases MMPs and angiopoietin coordinating the expression of the regulators of endothelial cell survival and modulators of cartilaginous destruction, the MMPs (135). In summary, the correct balance between growth factors and cytokines produced by endothelial cells and chondrocytes is essential (137) for the transition of cartilaginous callus into vascularised bone callus helping the physiological bone repair.

Interestingly, IL-17 can suppress chondrogenic differentiation of MSCs via down-regulate the expression of key chondrogenesis transcriptional factor, SRY-box 9 (SOX9) and its activator cAMP-dependent protein kinase (PKA) (138). Nam et al., have reported that an exposure of the osteoblasts to IL-17 increases the gene expression of collagen, osteocalcin and bone sialoprotein indicating enhanced maturation of osteoblasts (63). This together shows that Th17 cells constitute an important contributing factor into the transition of chondrogenesis into osteogenesis.

The summary of the repair phase

MSCs and other immune cells are involved in this stage (Figure 1 and 2). MSCs differentiate into chondroblasts developing cartilaginous callus, which then mature into chondrocytes, which help the mineralisation of cartilaginous matrix forming bone callus. The growth factors help the proliferation and the differentiation of MSCs as well as the maturation of chondrocytes. Additionally, macrophages help the deposition of cartilaginous matrix. Angiogenesis starts in this phase with a help of MSCs, macrophages and growth factors. Other immune cells, T and B lymphocytes reappear late in this phase during the mineralisation of

cartilaginous callus helping the transition into bone callus aided by inflammatory cytokines TNF-α and IL-17.

IV. Remodelling phase

Remodelling phase includes the reinstating of the normal architecture and the orientation of the growing bone to restore its normal function. This occurs by transforming of irregular woven bone callus into lamellar bone within two steps, the resorption of mineralised bone and then the formation of new lamellar bone (139, 140). This process is mainly dependent on the balance between osteoblast and osteoclast functions with the stimulation by BMPs (36). Also during this phase, new blood vessel formation is continued with minimal roles for immune and other hematopoietic cells.

i. Cells

The established role for MSCs during the bone healing is the differentiation into osteoblasts at later stages of the process (141). Osteoblast formation is tightly controlled by the influence of various growth factors and downstream signalling pathways. Under the effect of TGF-β, BMPs and IGF, MSCs differentiate into osteoblasts (142). Furthermore, the proliferation and osteogenic differentiation of MSCs is regulated via the activation of Wnt/catenin signalling pathway (143-145). Cyclooxygenase 2 (Cox-2) protein that induces the production of pro-inflammatory protein, PGE2 can also stimulate the differentiation of MSCs into osteoblasts. Cox-2 knock out mice show delayed healing of tibia fractures (146). Furthermore, reduced Cox2 expression with aging is associated with a delayed bone repair as shown in a mouse model of femoral fracture (147). MSCs have an inhibitory effect on osteoclast generation from monocytes progenitors via mechanism mediated partially by the production of Osteoprotegerin (OPG) (148). This shows how MSCs can control the late stages of the bone remodelling and further emphasizes the link between inflammation and osteogenesis.

Osteoblast progenitors mature into mid-stage of collagen-producing cells before fully maturing into osteoblasts, which also produce non-collagenous calcium and phosphate binding proteins such as osteocalcin and osteopontin forming mineralised bone (149). Differentiated osteoblasts are involved in the matrix formation in cbfa-1/Runx-2 and Osterix-dependent mechanisms causing the formation of new lamellar bone (114, 150). Osteoclasts are responsible for the bone resorption and they create acidic microenvironment to demineralise the bone matrix and produce enzymes, which erode the bone. The functions of osteoblasts are

balanced by that of osteoclasts and the disruption in this balance leads to defective bone formation. An increase in osteoclast cell numbers and RANKL have been detected in animal model of bone non-union (151).

It has been demonstrated that Th17 cells can enhance osteoclastogenesis (152). IL-17 enhances the differentiation of osteoblasts from MSCs as well as enhances the expression of RANKL on MSCs thus promoting osteoclastogenesis when MSCs co-cultured with peripheral blood mononuclear cells (PBMCs) (153) thus IL-17 can regulate the bone turnover. Although Th17 cells have not been detected in healing bone during remodelling, they are linked to osteoblast and osteoclast generation and function, highlighting their possible role in the final stage of the bone healing (154). Additionally, macrophages are suggested to be involved in the remodelling phase by enhancement of the osteoblast activity as well as the progenitors of osteoclasts (32). In total, inflammatory cells control the balance of osteoclasts and osteoblasts, which is the main feature of the remodelling phase.

ii. Soluble factors and Cytokines

The main soluble factors that regulate the process of mineralisation of cartilaginous callus are M-CSF, RANKL and OPG. Although the early peak of RANKL is mainly derived from T lymphocytes during the inflammatory stage, its second high expression is evident during the repair/remodelling phase (34). RANKL and M-CSF secreted by osteoblasts work to enhance the survival and the activity of osteoclasts (155, 156).

Additionally, a second elevation of pro-inflammatory cytokines, IL-6 and TNF-α has been shown to happen later as the mineralised callus is remodelled into lamellar bone. These cytokines that are increased later on healing, are expressed by MSCs and osteoblasts (34). Overall, certain cytokines and soluble proteins are released by active osteoblasts then these factors work to induce the generation of osteoclasts thus controlling bone remodelling.

The summary of remodelling phase of bone healing

Bone cells, MSCs as well as certain immune cells are the main players during this phase (Figure 1 and 2). During this phase, the hard callus formed of irregular woven bone is transformed into lamellar regular bone via maintaining the equilibrium between the activity of osteoblasts and osteoclasts. Controlling these processes, MSCs differentiate into osteoblasts and can promote osteoclastogenesis. Th17 and macrophages regulate the balance of osteoblast and osteoclast functions. Growth factors as well as cytokines, which produced by osteoblasts, induce the generation and the survival of osteoclasts.

V. Intramembranous healing

Intramembranous healing is characterised by the direct differentiation of MSCs into osteoblasts during the repair phase. Previous evidence has shown that certain immune cells are involved in this type of healing. Bone-lining resident macrophages contribute to the intramembranous bone healing as shown in mouse tibial fracture model (126). Furthermore, the effect of T lymphocytes seems to be extended to prepare the osteoblasts for the proliferative phase of the bone repair. It has been reported that lack of T lymphocytes in $RAG^{-/-}$ mice shows delayed maturation of osteoblasts and prolonged repair phase that further delay the remodelling phase (63). BMPs are essential for the proliferation of MSCs and the production of Alkaline phosphatase from MSCs and osteoblasts favouring bone formation (157, 158). BMP-3, BMP-4, BMP-7, and BMP-8 are strictly expressed when the osteoblastic recruitment is most active. BMPs transduce their signal through SMAD activation and in conjunction with other signalling pathways such as Notch pathway (159-161). These findings further demonstrate the strong link between the bone healing and immune response with a help of growth factors.

VI. MSC-immune cell interactions; implication in defective bone healing

Complications such as delayed or non-union of bone fractures could be related to systemic inflammatory diseases or fracture-related reasons including sepsis, severe soft tissue damage and multiple fractures (162). MSCs can be detected within non-union tissues but they have an impaired function including increased their senescence (163). Additionally, it has been demonstrated that the number and the proliferative capacity of BM MSCs can be impaired in patients with non-union fractures compared to healthy controls (164, 165). Furthermore, the osteogenic activity is lower compared to BM MSCs (166). However, these non-union MSCs seem to retain their osteogenic differentiation when activated in vitro (165, 167). Additionally, these non-union MSCs can regain a full capacity of osteogenesis if treated with BMP-2 (168). Although, it is unknown if the changes in MSC number and function are reasons or results of non-union, injecting autologous BM MSCs into non-union site is still widely used in therapy (169). Additionally, this indicates that the defect of non-union MSCs is related to microenvironment rather than being an intrinsic fault. Immune response is linked to bone haemostasis due to the direct effects of immune cells on osteoclasts and osteoblasts. Chronic inflammatory disorders due to systemic or local disease are always associated with bone

pathology. The effects of uncontrolled inflammation including cells and cytokines are reviewed below.

i. Neutrophils

A systemic activation of neutrophils using oxygen free radicals leads to a defective healing of bone fracture as shown in rodents (170). Additionally, an induced neutropenia in animal models of bone defects shows an enhanced osteogenic repair (171). Although it is not clear if neutrophils interact with MSCS, an excess activation of neutrophils has a negative effect on the bone healing.

ii. Macrophages

As shown in an experimental model of bone fracture in rat, an excessive activation of macrophages via systemic injection of lipopolysaccharide can reduce the secretion of BMP-2 by macrophages and results in a delayed bone healing probably via a negative effect on MSCs (157). Monocytes and particularly those which express a high level of a co-stimulatory molecule, Osteoclast-associated receptor (OSCAR) have a greater potential to differentiate into osteoclasts via TNF-α mediated mechanism within an excessive inflammatory milieu (172). This implies an adverse effect of activation of monocytes on the bone repair via their effects on both MSCs and osteoclasts.

iii. NK cells

Within an excessive inflammatory milieu, NK cells could play a role in the pathogenesis of delaying bone healing or even bone loss. Activated NK cells impair the survival and function of injected allogeneic or autologous MSCs and subsequently inhibit MSC-dependent therapeutic effects (102, 173-176). NK cells also have a similar role in the pathogenesis of inflammation-related bone destruction as shown in a mouse model of arthritis and the depletion of NK cells considerably prevents the bone erosion (47). In conclusion, activated NK cells could exert adverse effects on the bone haemostasis.

iv. T and B lymphocytes

A study comparing the abundance of cytotoxic T lymphocytes within the fracture hematoma between healing and non-healing groups of sheep has reported a predominance of T lymphocytes in the non-healing group (177). Moreover, a mouse model of gamma delta T cell knock out has been shown an improvement in the bone healing (178). Interestingly, the

uncontrolled stimulation of B and T lymphocytes appear to be important in delayed bone healing. $RAG^{-/-}$ mice, which lack T and B cells, show an enhancement of callus mineralisation and bone remodelling and acceleration of the fracture healing (42). These effects were associated with a lower level of pro-inflammatory cytokines, TNF-α, IFN-γ, IL-2 and IL-4 at local fracture tissues (42). Activated T lymphocytes are known to release RANKL, which enhance osteoclast differentiation from their progenitors and consequently induce the bone lysis (156). Similar to T lymphocytes, B lymphocytes can stimulate the generation of osteoclasts as they secret RANKL and specific autoantibodies in case of inflammatory arthritis. These autoantibodies have a high binding affinity to the surface of osteoclasts and their precursors inducing bone damage (179). Altogether, the excess activation of these adaptive immune cells is harmful for the bone generation.

In a recent study, a positive correlation has been observed between the delayed bone repair and increase of the expression of peripheral blood terminally differentiated $CD8^+$ $CD11a^+$ T lymphocytes, which highly produce IFN-γ and TNF-α (180). Moreover, a depletion of these $CD8^+$ T lymphocytes leads to an improvement of bone repair in an osteotomy mouse model (180). In another study, activated Th1 cells or cytotoxic T lymphocytes were found to block osteogenic differentiation of MSCs via the effect of IFN-γ (181). Interestingly, IL-17, which is produced by activated Th17 subset of T lymphocytes, has been linked to bone destruction in excessive inflammatory milieu (152). These studies confirm the link between effector T lymphocytes and impaired bone healing.

In contrast to effector subsets of T lymphocytes, T reg lymphocytes have shown no suppressive effect on MSC-mediated bone formation probably because of an associated suppressive effect on IFN-γ and TNF-α. This finding was further confirmed when injected T reg lymphocytes improved the MSC-mediated repair of bone fracture efficiently in an animal model (182). Transplantation of syngeneic MSCs induced T reg response, which was associated with a better healing compared to allogeneic MSCs, which induced Th1 response marked by the production of IFN-γ (181). Similarly, infusion of T reg lymphocytes in calvarial defect in mice was associated with an enhancement of MSC-mediated bone healing and a reduction of the expression of TNF-α and IFN-γ (181).

In conclusion, prolonged activation of immune cells inhibits osteogenesis. Depletion of haematopoietic cells from human allograft material containing live osteocytes (183) shows no adverse immune effects upon graft implantation in vivo (184), but it is critical to study such allograft's behaviour and MSC function generally in inflammatory milieu. This will pave the way to new therapeutic solutions for non-union bone fractures.

v. Cytokines

Prolonged or uncontrolled inflammatory phase of bone repair could be destructive. There is a link between excess inflammation e.g. in microbial infection, and impaired osteogenesis (185). Different inflammation-related cytokines and cells are linked to pathological bone healing. TNF-α mediated chronic inflammation in a diabetic mouse model has been shown to lead to the death of regenerating chondrocytes thus impairing the bone formation (186, 187). Importantly, TNF-α has been shown to stimulate the expression of Wnt signalling pathway antagonist, Dickkopf-1 (DKK-1) that has a direct suppressive effect on the differentiation of osteoblasts. Conversely, the suppression of DKK-1 has been shown to activate the Wnt signalling pathway and induce the bone growth (188). DKK-1 seems to suppress the OPG production and disturb OPG-RANKL balance, which leads to bone lysis (188). Another role for TNF-α is to suppress nephronectin, an extracellular matrix protein that induces the development and growth of osteoblasts (189). Additionally, TNF-α induces the production of M-CSF by BM MSCs, which in turn, enhance the expression of the key osteoclastogenic cytokine receptor, RANK in the osteoclast progenitors (190). Targeting TNF-α in these models reversed these effects and improved bone healing (187).

In chronic inflammatory milieu, TNF-α suppresses the biomarkers of MSC osteogenic differentiation via NF-κβ signalling-dependent mechanism (191). An exposure of mouse MSCs to high doses of TNF-α and IL-1 reduced their osteogenic differentiation as shown by suppression of ALP levels compared to controls (192). Both TNF-α and IFN-γ inhibited MSC osteogenic ability in dose-dependent manner in vitro (180). Interestingly, an in vitro exposure to IFN-γ augmented TNF-α-mediated apoptosis of BM MSCs and suppressed their osteogenic differentiation (182, 191). The IFN-γ-mediated inhibition of osteogenesis of MSCs was associated with increased expression levels of SMAD-6, a negative controller of bone differentiation (182). Additionally, IFN-γ enhanced the formation of osteoclasts (193). Another inflammatory cytokine, IL-6 mediates osteoclastogenesis, and blocking of IL-6 receptor was shown to protect from bone erosion in mouse model of arthritis (194). Similarly, osteoclast formation was significantly reduced in mouse model of arthritis where IL-1 was knocked out indicating that IL-1 has an important role in inflammation-mediated bone lysis (195).

The chronic inflammation and pathological bone formation are associated with dysregulation of BMPs and their inhibitors (35). The gene expression of BMP antagonists (noggin and follistatin) and certain MMPs, MMP-7 and MMP-12 are higher in non-union

tissues compared to that in normal healing callus (196). In contrast, many genes are inhibited in non-union including growth factors IGF-2, FGF-1, TGF-β2, PDGF, Wnt-induced proteins, B-catenin as well as receptors for PGE2 (197).

In summary, exaggerated or prolonged effect of inflammatory cytokines and cells have a negative effect on MSCs by inhibiting their osteogenic ability as well as a stimulatory role on osteoclasts leading to defective bone formation (Figure 3).

VII. MSC-Immune cell interactions; therapeutic implications to fracture repair

Animal studies and clinical trials, which used MSCs for therapeutic purposes of bone repair, indicated that the inflammation status could affect significantly the efficiency of these treatments. The magnitude of inflammation highly influences the immunomodulatory effects of MSCs (13). High dose of IFN-γ or other priming cytokines is needed to induce the immunosuppressive function of MSCs and consequently the bone healing. In contrast, weak inflammation or low dose of IFN-γ might not be enough to prime MSCs for immunosuppressive effect, instead, it could induce the antigen presentation property in MSCs and promote the recruitment of immune cells (198, 199).

Allogeneic MSCs derived from BM, placenta and umbilical cord and implanted under the kidney capsule on collagen-based matrix did not form ectopic bone compared to syngeneic MSCs in murine recipients (200). Similarly, cloned MSCs derived from Balb/c mice could not promote bone growth in allogeneic mice together with the suppression of osteogenic markers, Alkaline phosphatase, cbfa-1/Runx2 and osteocalcin (181). Additionally, the chondrogenic potential of synovial MSCs transplanted into allogeneic rat with anterior meniscus defect was impaired relative to autogenic implants (201). In these conditions, an increase of the local expression of NK cells, T and B lymphocytes as well as macrophages was detected (181, 201, 202). In addition, a high expression level of allo-specific antibodies was demonstrated in the recipient hosts (202). It has been proposed that Th1 response evident by increase of IFN-γ was responsible for the suppression of the bone growth (181). It is likely that the exposure of allogeneic MSCs to the activated host immune cells leads to suppression of the functions of these MSCs and failure of the therapy.

Interestingly, autologous MSCs are susceptible for killing by cytokine-activated NK cells. This killing is related to the low level of HLA class I and to the presence of ligands for

NK cell activating receptors on the surface of MSCs (102, 173-176). Also, the activation of NK cells against MSCs is related to specific adhesion molecules (176). In cases of GVDH, NK cells could have a negative effect on injected allogeneic MSCs, which threatenes the fate of the transplant (203). Despite these in vitro observations, it is still unknown if NK cell-mediated killing of allogeneic MSCs occurs when these MSCs used for therapy of the bone repair. In summary, NK cell-mediated killing of allogeneic MSCs could be another mechanism causing the failure of therapeutic use of MSCs.

Allogeneic and autologous BM MSCs loaded on hydroxyapatite or β-tricalcium phosphate scaffolds then implanted in femoral defect in dogs or tibia defect in mini pigs or radius injury in rabbits, showed similar extent of the bone growth without eliciting an immune response (204-206). Additionally, an implantation of adipose–derived MSCs loaded on matrix into ulnar defect in rabbits or added to fibrin glue to fill the mandible defect in rats showed positive bone healing results comparable to that using autologous MSCs (207, 208). Seeding of allogeneic MSCs in β-tricalcium phosphate scaffolds implanted in dogs has been shown to elicit an inflammatory response, measured by increase of the number of T lymphocytes, similar to that induced by autologous MSCs (209). These observations indicate that the interactions between allogeneic MSCs and recipient immune cells could be avoided if these MSCs are loaded on mineralised scaffolds thus minimizing their accessibility to host immune cells.

VIII. Conclusions and future perspectives

Osteoimmunology is an emerging discipline exploring the relationship between inflammation and bone biology and how inflammatory signals control bone healing. Similar to the repair of muscle injuries and skin wounds where MSCs are involved in all stages of the repair (210), MSCs interact with various cells, cytokines and growth factors during the whole process of the bone fracture healing (211). The effect of inflammation on the bone healing through interaction between MSCs and immune cells is complex. As explained, inflammatory cells and cytokines prime MSCs towards immunomodulatory function while the excess activation of the same mediators can suppress the osteogenic differentiation of MSCs. Furthermore, cytokines like IL-17 could promote or suppress osteogenesis via its dual effect on both osteoblasts and osteoclasts. Therefore a fine balance between the functions of MSCs and inflammatory mediators and cells as well as between osteoclasts and osteoblasts exists and the disturbance in

these balances can lead to the complications of the bone repair such as delayed healing or fracture non-union.

Whilst the suppression of inflammation is needed for MSC differentiation to proceed, inappropriate inflammatory-mediated priming of MSCs could lead to a failure in switching off the inflammatory signals and hence to impaired progression through the bone healing stages. The ultimate goal for using MSCs in cases of bone defect or fractures should not be only be as powerful osteogenic cells but also as immune mediators controlling the inflammatory stage of bone healing. Indeed, several therapeutic approaches targeting inflammation in animal models of delayed healing have been shown successful outcomes (212). The better understanding of MSC-immune cell interactions during the bone healing is therefore highly important in order to modulate MSC-based therapy and ultimately treat or prevent challenging cases of bone injuries.

Figure legends

Figure 1 Cells involved during three stages of secondary bone healing

MSCs are involved in the three phases of bone healing. Macrophages similarly play various roles during the whole process. Neutrophils and NK cells are known to be involved only during inflammatory phase. In contrast, B and T lymphocytes are involved in inflammatory phase then during late repair and early remodelling phase. Chondroblasts are specifically active during repair phase of the secondary healing. Osteoblasts are involved in the repair phase of the primary healing and in the remodelling phase of either secondary or primary healing. In comparison, osteoclasts are contributed in both inflammatory and remodelling phases of the repair.

Figure 2 Interactions between MSCs, immune cells and bone cells during bone healing

During inflammatory phase, immune cells induce the proliferation of MSCs and prime them. MSCs in turn, control inflammatory response. Additionally, immune cells stimulate osteoclasts to remove bone debris. During repair phase, MSCs differentiate into chondroblasts (secondary healing), which then mature into chondrocytes that form cartilaginous callus. With help of immune cells, cartilaginous callus converts into bone callus. Finally, balance between osteoblasts and osteoclasts help remodelling of bone callus into lamellar bone.

Figure 3 The magnitude of inflammation affects the fate of bone healing via interactions with MSCs

A short acute inflammatory phase carried out by active cells and cytokines, helps to recruit and stimulate the proliferation of MSCs. Additionally, this inflammatory response stimulates the immunosuppressive effects of MSCs ending the inflammatory phase and progressing into normal repair phase. In contrast, uncontrolled or chronic/exaggerated inflammatory phase is associated with excessively activated cells and cytokines. These factors lead to inhibition of proliferation and osteogenic differentiation of MSCs causing an impairment of bone healing.

Table 1: The role of immune cells during the bone healing

Immune Cell	Role	Phase	Reference
Neutrophils	Eliminate microbial infection. Remove damaged tissue and debris	Inflammatory Inflammatory	(30) (27)
Macrophages	Production of BMPs. Production of Oncostatin M that stimulates MSC osteogenesis. Exosomes stimulates MSC osteogenesis. Deposition of collagen I. Transition of cartilaginous callus into bone callus.	Inflammatory/Repair Inflammatory/Repair Inflammatory/Repair Repair Repair	(35, 36) (37, 38) (39) (126, 127) (120, 121)
NK cells	Priming of immunomodulatory function of MSCs. Migration of MSCs. Production of RANKL, which induces osteoclast formation.	Inflammatory Inflammatory Inflammatory	(42) (46) (47)
ILCs	IL-17: Osteoblast maturation, and osteogenesis of MSCs. IL-22: MSCs proliferation and differentiation.	Late inflammatory? Late inflammatory?	(63) (64)
T lymphocytes	Priming of immunomodulatory function of MSCs. Stimulate osteogenesis of MSCs. Production of RANKL, which induces osteoclast formation. Transition of cartilaginous callus into bone callus.	Inflammatory Inflammatory Inflammatory Late repair	(34) (71) (69, 70) (44, 45)
Th17 cells	Stimulate MSC osteogenesis. Induce osteoblast maturation. Transition of cartilaginous callus into bone callus. Induce osteoclast generation.	Inflammatory Repair Repair Remodelling	(63) (63) (138) (154)
T reg lymphocytes	Decrease immunosuppressive effect of MSCs Stimulate bone healing	Inflammatory Repair	(73) (72)
B lymphocytes	Stimulate osteoclastogenesis. Transition of cartilaginous callus into bone callus. Induce death of osteoclasts.	Inflammatory Late repair Early remodelling	(74) (75) (44, 45)

Table 2: The role of soluble factors during the bone healing

Soluble factor	Role	Phase	Reference
TNF-α	Migration of MSCs. Prime MSCs for immunosuppression. Regulate the functions of osteoclasts and osteoblasts	Inflammatory Inflammatory Remodelling	(87) (90) (44, 45)
IFN-γ	Stimulate proliferation of MSCs. Prime MSCs for immunosuppression.	Inflammatory Inflammatory	(92) (92)
IL-1	Prime MSCs for immunosuppression.	Inflammatory	(91)
IL-17	Prime MSCs for immunosuppression. Stimulate the proliferation and osteogenesis of MSCs. Promote osteoblast maturation. Induce osteoclastogenesis.	Inflammatory Repair Remodelling Remodelling	(95) (63) (63) (153)
IL-22	The migration, proliferation and differentiation of MSCs	Inflammatory	(64)
CCR2	Increase vasculature. Recruit macrophages.	Inflammatory And repair	(31)
TGF-β	Suppress immune response. Enhance osteogenic differentiation of MSCs.	Inflammatory Repair	(96, 97) (96)
BMPs	Enhance proliferation and osteogenic differentiation of MSCs. Promote osteoblast maturation.	inflammatory/Repair Repair	(157, 158) (159-161)
SDF-1	Recruitment of MSCs	Inflammatory	(83)

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