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Bone Microenvironment

Yingying Jing[1] *and Xiao Chen*[2]

[1] *Shanghai University, Institute of Translational Medicine, No. 333, Nan Chen Road, Shanghai, 200444, China*

[2] *Changhai Hospital, Department of Orthopedics, Shanghai, No. 168, Chang Hai Road, 200438, China*

1.1 Introduction

1.1.1 Cell Types

There are many types of cells in bone microenvironment [1], including genuine bone cells (osteoblasts, osteocytes, osteoclasts, and their precursors), cells of the hematopoietic and immune systems, stromal cells, adipocytes, fibroblasts, and endothelial cells and so on [2]. A growing body of evidence, with the development of techniques such as single-cell sequencing, proposes a fluidity in the ability of bone marrow (BM) stem cells to differentiate toward distinct lineages [3]. In this section, the main cells in the bone microenvironment are presented below with origins, functions, and identifications.

1.1.1.1 Genuine Bone Cells

1.1.1.1.1 Bone Mesenchymal Stem Cells

As defined by the International Society for Cellular Therapy (ISCT), mesenchymal stem cells (MSCs) are capable of adhering to plastic and capable of differentiating toward adipogenic, osteogenic, and chondrogenic pathways and other specific phenotypes [4]. In the bone marrow, the percentage of MSCs is very low in terms of numbers, only 0.01% [5], but these cells play an important role, especially CAR cells (CXCL12-rich reticulocytes), CD146+ cells, and Nestin+ cells [6]. CAR cells are a subtype dispersed within the bone marrow that regulates the cell cycle and hematopoietic stem cell (HSC) self-renewal through high expression of CXCL12 and SCF [7, 8]. CD146+ cells are a subtype predominantly found in the human vascular ecology that interacts with HSCs and endothelial cells through the expression of Tie-2 and CXCR4 [9]. Nestin+ cells are associated with the nerves of the sympathetic nervous system (SNS) [6, 10] in the perivascular area of bone marrow [11]. It supports the homing role of HSCs and also regulates homeostasis of HSCs by

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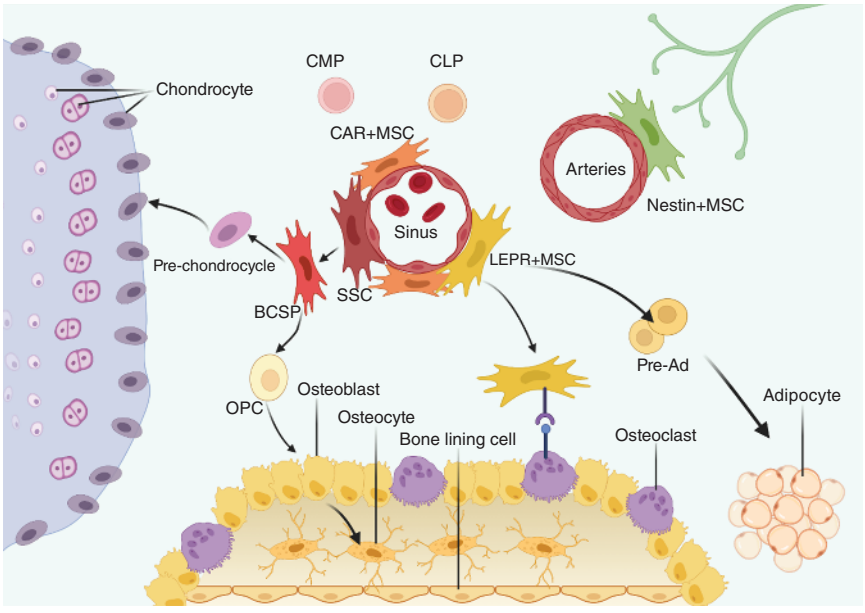

Figure 1.1 Classification of BMSCs. Source: Adapted from Gao et al. [15].

maintaining high expression of various genes such as CXCL12, SCF, and Ang1 [11]. Besides, skeletal stem cells (SSCs) have been identified as a lineage-restricted subset of bone marrow mesenchymal stem cells (BMSCs) with self-renewal and osteochondral properties [6, 12]. They are able to differentiate into osteo-lineage cells, bone, cartilage, and stroma [13, 14] (Figure 1.1).

1.1.1.1.2 Osteo-Lineage Cells

Osteoblasts include osteogenic progenitor cells, preosteoclasts, and osteoblasts. It is now accepted that the whole process can be divided into three distinct stages of differentiation. In the first stage, the transition in osteoblasts to pre-osteoblasts is marked by the expression of RUNX2 in osteoblasts. In the second stage, WNT-β-catenin signaling acts on pre-osteoblasts to induce Ostrix expression. In the third stage, the expression of both RUNX2 and Ostrix drives differentiation toward osteoblasts [16]. Osteoblasts are located between the bone matrix, and they are derived from a subpopulation of osteoblasts [17].

Osteoblasts secrete extracellular matrix proteins, such as type I collagen, periostin, osteocalcin, and alkaline phosphatase. Among them, type I collagen plays an important role in bone mineralization by depositing calcium together with the hydroxyapatite form. Moreover, the mechanism of mutual coupling between osteoblasts and osteoclasts maintains bone mass homeostasis. The process of bone maintenance is sensitive to mechanical forces, and in response to mechanical loading, osteoblasts lead to increased bone formation by activating multiple signaling pathways, mainly the WNT-β-catenin signaling pathway [18]. There are other conditions such as radiation and diet that also have an impact on osteoblast function [19–21].

1.1.1.1.3 Bone Lining Cells

Bone lining cells are also differentiated from osteoblasts [22]. In general, bone lining cells are defined as elongated, flattened cells on the bone surface in areas where no bone remodeling activity occurs [23]. Bone lining cells, similar to osteoblasts, express some level of alkaline phosphatase. However, bone lining cells phenotypically express intercellular adhesion molecules, but not osteocalcin, which is the major difference between them and osteoblasts [24].

Recent studies have shown that bone lining cells play an important role in bone remodeling. They communicate with osteoblasts deep in the bone matrix through gap junctions and regulate the transformation of HSCs between the undifferentiated state and osteoblasts under different conditions.

In addition, before bone-forming activity, bone lining cells first remove osteoclast remnants of matrix-by-matrix metalloproteinases [25], such as demineralized collagen [26]. Afterwards, osteoblasts can then enter the site to deposit new bone [27].

1.1.1.1.4 Osteoclasts

Osteoclasts are special cells from the monocyte/macrophage hematopoietic lineage, and morphologically, they are multinucleated cells. Their main hallmark is the expression of high levels of tartaric acid-resistant acid phosphatase and cathepsin K [28]. Osteoclasts play an important role in the coupling of bone formation to bone resorption through the RANK signaling pathway [29].

1.1.1.2 Chondral-Lineage Cells

Chondrocytes are cells that produce and maintain the cartilage matrix and characteristically express the SOX gene [30]. Prechondrocytes develop from MSCs, which later differentiate into chondrocytes. Growing chondrocytes continue to undergo cell division as they grow, and the divided daughter cells usually form cell clusters distributed in the cartilage matrix. In contrast, older chondrocytes have a basophilic cytoplasmic nature due to an increase in the rough endoplasmic reticulum [31]. Chondrocytes release extracellular matrix and collagen fibers to form elastic and collagen fibers [32].

1.1.1.3 Adipocytes

Adipocytes are abundant and occupy a large amount of space in bone marrow. The types of adipocytes include preadipocytes and mature adipocytes [31]. Adipose precursor cells are a specialized class of cells that do not contain lipid droplets but express adipocyte markers. They are usually present in large numbers in the perivascular area, especially in the intraosseous veins, and are not proliferative. They can maintain vascular function and inhibit bone formation by occupying space [33]. In addition, it is noteworthy that adipocytes have been found to be associated with many pathophysiological mechanisms [34]. For example, preadipocytes and mature adipocytes can recruit multiple myeloma cells via monocyte chemotactic protein-1 and stromal cell-derived factor-1α and produce many secreted factors that support multiple myeloma cells in the bone marrow [35].

1.1.1.4 Cells of the Hematopoietic Systems

1.1.1.4.1 Hemopoietic Stem Cells

HSCs produce billions of new blood cells every day and are responsible for the continuous renewal of blood. It is generally acknowledged that HSCs can further differentiate into two main types: common lymphoid cells and common myeloid cells [36]. HSCs can be obtained from umbilical cord blood, bone marrow, and adult peripheral blood. The most primitive human HSCs were identified as CD34+CD90+Lin- [37]. Depleted expression of CD45RA has also been used in combination with the above markers to identify primary HSCs [14]. Most HSCs are in a resting state and are activated upon external stimulation [38].

1.1.1.4.2 Lymphoid Cells

Common lymphoid progenitor cells are differentiated from HSCs stimulated by IL-7 [39]. Further, stimulated by cytokines such as IL-3 and IL-4, lymphoid progenitor cells differentiate into B lymphocytes [40]. Once maturation, B cells enter the circulatory system and eventually localize in the lymphoid follicles of peripheral lymphoid organs [41]. B cells are one of the major specific immune cells, accounting for 20% of peripheral lymphocytes [42]. In addition, lymphoid progenitor cells differentiate into natural killer (NK) cells in response to IL-15 stimulation [43].

1.1.1.4.3 Myeloid Cells

Common myeloid progenitor cells are differentiated from HSCs in response to stimulation by IL-3, GM-CSF, and M-CSF [44]. Myeloid progenitor cells can differentiate in two directions, toward granulocyte-macrophage progenitors and megakaryocyte-erythroid progenitors, depending on the stimulating factors in the bone microenvironment.

The megakaryocyte-erythroid progenitor cells are stimulated by erythropoietin to produce erythrocytes, the most abundant blood cells in the blood and the main mediator of oxygen transport through the blood in vertebrates, and also have immune functions. Stimulation by IL-3, IL-3, SCF, and TPO results in the production of megakaryocytes by megakaryocyte-erythroid progenitor cells. Megakaryocytes are a type of cell in the bone marrow that form platelets after partial rupture in response to IL-11 and TPO stimulation. Platelets play an important role in bleeding and clotting processes [45].

Granulocyte-macrophage progenitor cells can differentiate into primitive granulocytes and monocytes. They differentiate into monocytes under the stimulation of GM-CSF and M-CSF. Monocytes differentiate into macrophages under the stimulation of IL-6, IL-10, M-CSF, and IFN-gamma [46] (Figure 1.2).

1.1.1.5 Cells of the Immune Systems

Research related to immune cells in the bone microenvironment has gradually entered the osteopathic field in recent years. Bone health is affected by them in various ways, including the immune effects of immune cells and immune factors themselves and the regulation of the bone microenvironment.

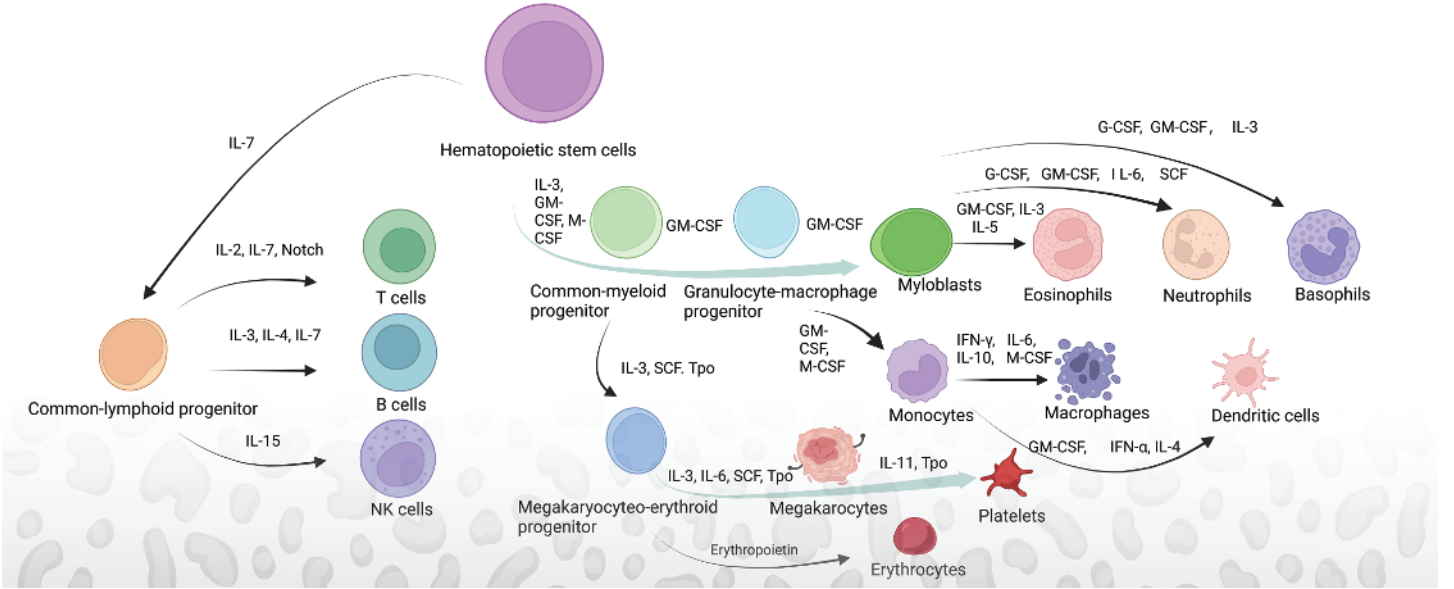

Figure 1.2 The differentiation spectrum of human HSCs. Source: Chen et al. [47]/with permission of Elsevier.

1.1.1.5.1 T-Cells and Natural Killer Cells

T cells not only play a key role in adaptive immunity, but are equally significant in bone immunology. Basically, all T-cell subtypes have some impact on osteoblasts (mainly osteoclasts). Nevertheless, current studies have identified a capable role for Th17 cells in inducing osteoclast genesis. They characteristically express the cytokines: IL-17A, IL-17F, IL-22, IL-26, and IFN-γ [48]. In osteoblasts and stromal cells, it can also induce the expression of macrophage colony-stimulating factor (M-CSF) and RANKL expression [49].

Most NK cells have similar osteoimmune functions to lymphocytes. Recent studies suggest that NK cells may be a target for rheumatoid arthritis (RA) treatment, based on the observation of osteoblast death by NK in RA-induced bone destruction [50, 51].

1.1.1.5.2 Dendritic Cells

Dendritic cells (DCs) are antigen-presenting cells with the specific role of guiding immune cells to the correct target as soon as possible and avoiding autoimmunity [52]. In fact, they have an indirect role in bone immunity mainly by presenting antigens of T cells [53]. Cytokine signaling about DCs can also regulate their activities and subtype homeostasis [54, 55]. On the other hand, in RA, DCs can be transdifferentiate into osteoclasts in response to stimulation by M-CSF and RANKL as if they are precursor cells for osteoclasts [56].

1.1.1.5.3 Neutrophils

Neutrophils have a strong presence in bone loss caused by particular inflammation [57]. In the presence of inflammation in systemic tissues, including bone tissue, neutrophils are usually the first to migrate to the site of injury [58] and secrete chemokines, cytokines, and small molecules that are capable of acting as immunomodulators. Most of the current studies task that activated neutrophils directly or indirectly induce osteoclast genesis [59].

1.1.1.5.4 B Cells

B cell production and development are dependent on factors produced by cells in the bone marrow stroma, such as RANKL, OPG, IL-7, and CXCL12 [60, 61]. B cells themselves produce RANKL [62] since conditional knockdown of RANKL in B lymphocytes can partially counteract the increased number of osteoclasts, thereby protecting against ovariectomy-induced bone attrition. Interestingly, no effect was observed in T lymphocytes [63]. This suggests the existence of a specific role of B lymphocytes on osteoclasts.

1.1.1.5.5 Osteomacs and Macrophages

Bone marrow macrophages and osteal macrophages, also called bone macrophages, are the resident macrophages in bone and, like many other organs, play a long-term immune role in the corresponding organ [64]. *In vitro* and *in vivo* studies have shown that these bone macrophages play a role in osteoblast differentiation through the production of bone morphogenetic proteins (BMPs) [65] and Oncostatin M [66].

Furthermore, elimination of bone macrophages inhibits further differentiation of primary osteoblasts [67]. If periosteal macrophages are selectively ablated, young mice show reduced bone growth and osteoporosis [68]. Thus, bone macrophages are cells with pleiotropic functions, both in regulating bone mass and in becoming osteoclasts, as well as actively participating in the homeostasis of the immune system.

1.1.2 Extracellular Matrix

In the bone microenvironment, the ECM is involved in regulating various cell behaviors, responses to growth factors, and differentiation. The recent spurt of research on the osteoinductive, osteogenic, and osteogenic potential of ECM-based scaffolds has advanced the rapid development of regenerative orthopedic medicine. ECM-modified biomaterials and decellularized ECM scaffolds are two types of scaffolds that are widely used for bone tissue engineering [69].

1.1.2.1 Inorganic ECM

The main inorganic component of hard tissues in the body, such as bones and teeth, is hydroxyapatite (HA, $Ca_5(PO_4)_3OH$) [70]. The usual biomineralization process, referring to the series of physiologically regulated activities occurring in the bone microenvironment culminates in the deposition of HA. The template for HA deposition is collagen, which is mostly expressed and secreted into the bone matrix by osteoblasts [71].

1.1.2.2 Organic ECM

1.1.2.2.1 Collagenous Proteins

Type I, type III, and type V collagen constitute the richest components of bone in terms of organic ECM. The primary function of collagen is to provide mechanical support and to act as a scaffold for bone cells [72]. The molecular structure of type I collagen is a triple-helix polypeptide of collagen fibrils. Together with other collagen and non-collagenous proteins, these fibrils are assembled into fibrils bundles and higher order fibers [73]. Type III and V collagens are less abundant. Their function is to participate in the formation of collagen bundles as described above [74]. Inter- and intra-chain cross-links of collagen form a tight fibrous structure to maintain bone strength. A deficiency of collagen or a mutation in its structure can greatly alter the ECM and thus greatly increase the risk of fracture [75].

1.1.2.2.2 Noncollagenous Proteins

Proteoglycans Proteoglycan is a structure in which glycosaminoglycan (GAG) residues are covalently bound to proteins. Its species include heparan sulfate, hyaluronic acid, keratin sulfate, chondroitin sulfate, and dermatan sulfate [76]. In the bone microenvironment, small leucine-rich proteoglycans (SLRPs) are the most important proteoglycans. SLRPs are involved in all steps of the bone formation process such as cell proliferation, osteogenesis, mineral deposition, and bone remodeling [77]. In addition, SLRPs regulate collagen fibrosis, and their dysregulation eventually leads to fibrosis caused by orthopedic injury or genetic defects [78].

γ-Carboxyglutamic Acid-Containing Proteins γ-Carboxyglutamic acid (Gla) is a glutamate produced by vitamin K-dependent post-translational modifications appearing in bone matrix and other calcified tissues [79]. Osteocalcin (OCN), matrix Gla protein (MGP), and periosteal proteins in bone all contain Gla protein [80]. OCN is an important player in osteoblasts performing bone formation and bone reconstruction functions. It contains three Gla residues that regulate calcium metabolism by binding to hydroxyapatite [81]. MGP, on the other hand, regulates the synthesis of osteoblasts, osteocytes, and chondrocytes in the skeleton. It has been reported that bone mineralization is advanced in MGP-deficient mice [82, 83]. In contrast, intramembranous bone mineralization is reduced in mice overexpressing MGP [84]. In addition, periostin, another Gla-containing protein, is abundantly expressed by osteoblasts in long bones and is involved in collagen folding and fibrillogenesis [85].

Glycoproteins Glycoproteins have different combinations and positions in the protein chain where covalently linked glycoprotein molecules exist. Among the glycoproteins of the bone microenvironment, osteoprotegerin is the most common and capable of bone mineralization. Osteoblasts highly express osteoprotegerin and secrete it into mineralized tissues. Osteoprotegerin regulates calcium deposition by binding collagen crystals and hyaluronic acid [86]. In the bone microenvironment of the developing skeleton, thrombospondins (TSP), grouped from TSP1 to TSP5, play an important role. One study reported increased bone mass and thickness of cortical bone and promotion of differentiation of osteoblasts in TSP1-deficient mice, possibly due in part to potential TGF-β activation [79]. R-spondins (parietal plate-specific spondins) are four secreted, homologous glycoproteins belonging to a family of matricellular proteins with a TSP repeat sequence [80]. They are widely expressed in all developmental stages of skeletal tissue and act as an enhancer of the Wnt/β-catenin signaling pathway through leucine-rich repeat sequences 4, 5, and 6 of the G protein receptors (Lgr4/5/6). R-spondin is thought to be a skeletal regulator that controls embryonic bone formation and adult bone remodeling [81].

Small Integrin-Binding Ligand N-Linked Glycoproteins/SIBLINGs A kind of glycophosphoproteins generally found in mineralized tissues, named SIBLINGs, consist of bone sialoprotein (BSP) and osteocalcin (OCN) [87].

First, BSP is a glycosylated, non-collagenous phosphoprotein that is expressed at the onset of hard connective tissue mineralization. It has been shown that mice deficient in BSP have significantly diminished bone deposition and a significantly reduced rate of bone formation, resulting in a decrease in both the length of long bones and the thickness of cortical bone. Thus, BSP has an important function in regulating osteoblast differentiation and initiating bone mineralization [88].

OPN is also an important regulator highly expressed by osteoblasts, bone lineage cells, especially in bone transformation. OPN is enriched in sites that undergo phosphorylation during inhibition of mineralization, such as serine, acidic, and aspartate patterns. In addition, in bone remodeling, OPN is involved in regulating osteoclast production and osteoblast activity [89] (Figure 1.3).

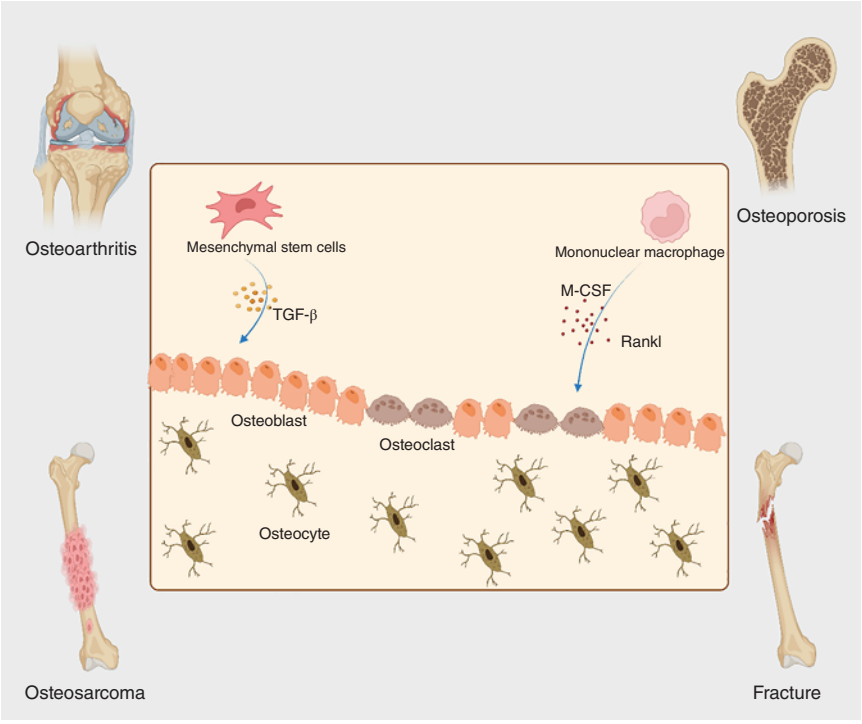

Figure 1.3 Bone microenvironment and diseases.

1.2 Bone Microenvironment and Diseases

1.2.1 Bone Microenvironment in Osteoporosis

The bone microenvironment consists of complex structures and biological systems, including bone cells (BMSCs, osteoblasts, osteoclasts, bone cells, and their precursors), fibroblasts, adipocytes, hematopoietic cells, immune cells, endothelial cells, and a large number of growth and signal factors in extracellular matrix [90]. Proper bone homeostasis maintenance relies on the equilibrium between bone formation and bone resorption. However, patients suffering from osteoporosis have the characteristics that bone resorption is greater than bone formation, which leads to bone loss and fragility-related fracture [91].

1.2.1.1 Bone Marrow Mesenchymal Stem Cells (BMSCs) and Osteoporosis

BMSCs in patients with osteoporosis show changes in osteogenic ability. It was found that the transcriptome of BMSCs in the bone microenvironment of elderly patients with osteoporosis changed compared to that in elderly patients without osteoporosis [92]. The levels of MAB21L2 and SOST expressed by BMSCs in osteoporosis were remarkably increased, which were inhibitors of BMP transcription and Wnt signal, respectively. BMSCs from patients with osteoporosis expressed higher

levels of genes related to osteoclastogenesis, which indicates that their osteogenic ability is limited. At the same time, they enhanced the production of osteoclasts through local release factors. Multiple *in vitro* studies have found significantly reduced proliferative activity (reduced S-phase fraction) and differentiation potential (reduced Osterix [Osx] expression and alkaline phosphatase [ALP] activity) and enhanced expression of aging markers in aging mouse BMSCs [93]. Recent experiments have shown that microRNAs in BMSCs of patients with osteoporosis have also changed. For example, overexpression of miR-21 in BMSCs can enhance osteogenic differentiation and bone formation [94].

BMSCs from patients with osteoporosis showed decreased response to anabolism irritant. As mentioned above, BMSC osteoblasts induced by 25 (OH) D3 are weakened in cells from elderly donators, and a coordination dosage of PTH is required to restore this reaction [95]. BMSCs from elderly subjects showed decreased expression and activity of CYP27B1, which was increased in PTH treatment. In recent experiments, compared with young people, the higher levels of PEHR and CREB activation expressed by BMSCs in the bone marrow of the elderly have been more stable β-Catenin induced by PTH [96]. Mice knockout of IGF-1 in BMSC showed a decrease in bone mass. Interestingly, IGF-1 in BMSC decreased in osteoporotic mice, suggesting that IGF-1 in BMSC is related to the occurrence of osteoporosis [97]. BMSC in bone microenvironment has undergone many changes in patients suffering from osteoporosis relative to healthy person.

1.2.1.2 Osteoblasts and Osteoporosis

Senile osteoporosis is caused by insufficient osteoblast function, while postmenopausal osteoporosis is mainly caused by an increase in bone resorption activity of osteoclasts due to estrogen deficiency. Various local and systemic factors under physiological and pathological conditions can affect the strict coupling activities of osteoblasts and osteoclasts, leading to the imbalance of bone remodeling and promoting bone resorption. Moreover, the change in osteoblast function plays a significant function in the occurrence of osteoporosis. Abundant experimental studies show that under the condition of osteoporosis, compared to normal osteoblasts, osteoblasts have low proliferation ability and defective function.

Long-term use of glucocorticoids is a prime reason of osteoporosis. High-dose and long-term glucocorticoid stimulation inhibited the proliferation and activity of osteoblasts and promoted the apoptosis of osteoblasts. At the same time, it increases the expression of RANKL, reduced the production of OPG, and enhanced bone resorption [98]. *In vitro* studies showed that dexamethasone treatment of human osteoblasts could overexpress DKK-1 mRNA. This indicates that glucocorticoids can inhibit Wnt signal transduction and inhibit osteogenesis. Glucocorticoid can reduce the expression of BMP-2 and increase its antagonist follistatin to inhibit osteogenesis [99]. PTH and bisphosphonates for the treatment of osteoporosis can change the difference of dexamethasone on BMP and Wnt signal transduction. Interestingly, pretreatment of BMSCs in elderly subjects with dexamethasone increased the expression of PTHR1 and saved the defect of proliferation induced by hormone.

1.2.1.3 Osteoclasts and Osteoporosis

Osteoclast is a highly differentiated multinucleated giant cell, which is the main functional cell for bone tissue resorption and participates in the process of bone remodeling throughout life. Postmenopausal osteoporosis patients produce more osteoclasts by increasing hematopoietic progenitor cells and increasing the recruitment of more osteoclast progenitor cells due to estrogen deficiency. The upregulated expression of osteoclasts leads to the expansion of cortical porosity and absorption regions indicated by bone trabeculae. At the same time, the life of osteoclasts in the bone microenvironment increases, which further prolongs the time of bone loss, deepens the absorption cavity, and increases the brittleness of bone [92].

Some animal experiments have shown that the cytokine tumor necrosis factor-alpha (TNF-α), which is mainly secreted by macrophages can increase osteoclast production in ovariectomized mice. TNF and RANKL synergistically increased the differentiation of hematopoietic pluripotent stem cells into osteoclasts, thus increasing the production of osteoclasts [93]. IL-6 is increased in the bone microenvironment of patients with osteoporosis. IL-6 is widely considered to be an effective stimulator of osteoclast-driven bone resorption. *In vivo* studies showed serious damage to cortical and trabecular bone microstructure, increased osteoclast production, and decreased osteoblast production in transgenic mice overexpressing IL-6 [94]. The important role of IL-6 is to promote the expression of signaling molecules downstream of osteoblasts, such as RANKL, so as to enhance the formation and activity of osteoclasts. In addition, IL-6 increases the promoting effect of IL-1 and TNF on bone resorption by increasing the pool of osteoclast progenitor cells. IL-7 is a major osteoclast factor, which stimulates T cells to produce RANKL and TNF and promotes bone loss. IL-7 can stimulate the increase in TNF in T cells. Moreover, the expression of IL-7Rα and IL-7 was upregulated by TNF. Therefore, there may be an interaction mechanism between TNF and IL-7 [95].

1.2.1.4 Bone Marrow Adipocytes (BMAs) and Osteoporosis

The accumulation of fat in the bone marrow of osteoporosis patients increases. Studies have shown that bone marrow adipocytes (BMAs) can inhibit bone formation and hinder fracture healing, and their content is negatively correlated with bone mass [100, 101]. Bone formation was also enhanced when BMAs decreased. After ablation, BMAs promote the recruitment and differentiation of pre osteoblasts into mature osteoblasts [102]. Recent studies have found that BMA can inhibit the function of osteoblasts by producing IL-6 [103, 104]. BMAs mediate myeloma induced inhibition of osteoblast formation. Multiple myeloma is characterized by excessive bone resorption and impaired osteogenesis [105]. When BMSCs were cultured with conditional medium from myeloma patients with BMA or pre-exposed myeloma cells, researchers observed reduced alizarin red S staining, alkaline phosphatase levels, and osteoblast gene expression [106]. BMA and osteoblasts are derived from BMSCs. When the adipogenic differentiation of bone MSCs increases, the osteogenic differentiation will decrease. Therefore, according to the above characteristics, osteogenesis can be changed by changing the differentiation direction of BMSC, so as to change the bone mass [107, 108]. BMAS promote osteoclastogenesis [109]. Adipoq^{cre};

$Rankl^{fl/FL}$ mice showed similar BMAs amplification compared with control Ranklfl/FL mice, but the number of osteoclasts decreased [110].

1.2.2 Bone Microenvironment in Osteoarthritis

Osteoarthritis (OA) is the most common joint disease with predominant damage to articular cartilage and involvement of the entire joint tissue, eventually leading to degeneration, fibrosis, fractures, defects, and damage to the entire joint surface. It is characterized by joint pain, stiffness, hypertrophy, and limited movement, and it occurs most commonly in weight-bearing joints such as the knee.

1.2.2.1 Subchondral Bone and Osteoarthritis

1.2.2.1.1 Subchondral Bone Cells

Subchondral osteoblasts are derived from BMSCs. Osteoclasts are not only involved in bone resorption in subchondral bone metabolism, but they also play an important role in the formation of H-type vessels. The histone proteinase K (ctsk) expression of anti-tartrate acid phosphatase positive (trap+) cells located around the cartilage-bone junction was lower than that of cells in the bone marrow interstitial space and had fewer nuclei [111]. Vascular-associated osteoblasts have a high affinity for H-type vessels, whose endothelial nuclear factor-κ B ligand (RANKL) expression is supported by receptor activators and induces H-type vascular anastomosis.

High turnover rate. The relative balance of bone formation and bone resorption establishes a stable microenvironment of subchondral bone. The subchondral bone conversion rate changes in response to changes in mechanical stress to maintain a stable microenvironment. The subchondral bone structure and mechanical support were abnormal in patients with OA. The number of osteoclasts in the bone microenvironment increases. Interestingly, osteocytes and chondrocytes provide the main RANKL. Abnormal mechanical force activates RANKL signaling to promote osteoclast fusion differentiation that promotes osteoclast formation, resulting in bone remodeling [112]. Soluble RANKL can pass through the subchondral bone plate cavity, which can promote the maturation of osteoclasts and play the role of bone resorption. It is found that subchondral bone plays an important role in cartilage injury [113]. Significant increase in osteoclasts near perichondrium trabeculae in subchondral bone marrow [114]. The subsequent remodeling process is related to the growth of blood vessels and nerves and the activity of osteoclasts. This suggests that during the onset of OA, subchondral bone undergoes increased bone remodeling conversion in response to external stimuli.

1.2.2.2 Cartilage and Osteoarthritis

1.2.2.2.1 Cartilage Erosion

There is top-down erosion from synovial tissue and synovium in the process of cartilage vascular invasion in patients with OA, but the most important is the bottom-up vascularization of subchondral bone. Matrix digestive proteases, such as MMPs, play an important role in angiogenesis. Subchondral angiogenesis plays a key role in cartilage degradation. In the process of cartilage formation, endothelial cells express

more MMP-9. Knocking out MMP-9 endothelial cells will lead to the destruction of bone formation ability and the formation of abnormally large bone plates, which indicates that MMP-9 is pivotal to bone resorption during cartilage formation. When vascular endothelial growth factor (VEGF) is injected into the joints of rabbits, it accelerates the formation of arthritis, and the use of inhibitors of VEGF can protect the articular cartilage [115, 116]. These experiments further suggest that neovascularization has a cartilage resorption effect on OA and endochondral ossification.

1.2.2.2.2 Mechanical Stimulation and Cartilage Homeostasis

Proper mechanical stimulation can maintain the health of articular cartilage. Overload will lead to cartilage fissure and bone atrophy. Research shows body weight, especially in obese individuals, weight load, and daily knee activity are associated with cartilage degeneration, and the knee is more prone to degenerative disease on the medial side [117]. In chondrocyte impact experiments, early mitochondrial dysfunction followed by cell death occurred in chondrocytes, but chondrocytes in the weight-bearing zone were less likely to die [118]. Appropriate biological load not only promotes the formation of cartilage matrix but also promotes the synthesis of matrix protein, collagen, and GAG. However, when the load is too heavy, p38 will be hyperphosphorylated, and MMP-13 will be overexpressed, resulting in matrix degradation and proteoglycan loss [119]. In the presence of increased circulatory pressure *in vivo*, the differentiation of BMSC toward the osteogenic aspect of formation increases due to various effects. MSCs under abnormal stress showed increased angiogenesis [120]. There are several cytokines closely related to angiogenesis in the above medium, such as FGF, TGF-β, and MMP-2. Other studies have shown that BMP-dependent signaling promotes osteogenic differentiation [121]. Thus, as described above, MSC from sclerotic subchondral bone may promote angiogenesis, thereby promoting cartilage degradation.

1.2.3 Bone Microenvironment in Fracture

Bone has the ability to regenerate. Fracture healing restates the mechanism of bone tissue formation in embryogenesis. In this way, fracture healing can restore the original structure and function rather than scar formation. Fracture healing can be divided into intramembrane osteogenesis and endochondral osteogenesis. Endochondral osteogenesis is to form cartilage callus in the area between medullary cavity and cortex and then form new bone through endochondral ossification. In conclusion, the stages of fracture healing are divided into a period of hematoma inflammatory response, cartilage scab formation, hard bone scab formation, and remodeling. Hematoma is a fibrin clot formed by coagulation at the injured bone, with infiltration of mast cells and other inflammatory cells. With the infiltration of fibroblasts and endothelial cells, granulation tissue was formed and gradually degraded to replace hematoma. Chondrocytes produce cartilage matrix and transform granulation tissue into cartilage callus. With the osteoid deposition of hydroxyapatite calcium and osteoblasts, the callus became woven bone. After rearrangement of collagen fibers, bone formation and differentiation of osteoblasts occur.

1.2.3.1 Cells in Bone Microenvironment

1.2.3.1.1 Platelet

Platelets are formed from cytoplasmic fragments of megakaryocytes in bone marrow. After vascular injury, platelets interact with collagen, von Willebrand factor (VWF), and fibronectin under the damaged endothelium to mediate the adhesion and activation of platelets. Platelets can be activated after endothelial cell damage. The particles released by platelets include dense particles α particles and lambda particles [122]. Platelet granules contain many cytokines related to osteogenesis and angiogenesis, and these cytokines play an important role in the healing process of fracture fractures [123]. These cytokines promote the chemotaxis and vascularization of inflammatory cells and the differentiation of BMSC to osteoblasts [124–126]. Among these cytokines, PDGF-AB and TGF-β can promote the proliferation and migration of vascular smooth muscle cells. During the inflammatory phase of the hematoma, growth factors can promote the formation of blood vessels and collagen and subsequently support bone healing. TGF-β1 can inhibit the formation of osteoclasts, PDGF-AB supports the proliferation of smooth muscle cells, and both the above growth factors promote collagen synthesis and angiogenesis to support bone healing [127, 128]. In addition, platelet-rich plasma can increase the production of bone morphogenetic protein 2 (BMP-2) from MSC, so as to comprehensively improve the bone regeneration of bone defect [129].

1.2.3.1.2 Erythrocytes

Erythrocytes are binucleate concave cells. The hematoma formed in the early stage of fracture mainly contains erythrocytes, platelets, and leukocytes. The number of each group of cells in the hematoma formed at the initial stage of fracture change dynamically. The erythrocytes in the hematoma decreased significantly from the fourth day of fracture, which was due to MSC proliferation and inflammatory cell infiltration in the hematoma. The number of each group of cells in the hematoma formed at the initial stage of fracture changed dynamically. The erythrocytes in the hematoma decreased significantly from the fourth day of fracture, which was due to MSC proliferation and inflammatory cell infiltration in the hematoma. The interaction between erythrocytes and platelets contribute significantly to the process of coagulation. The negative charge of phospholipids in erythrocyte membrane is related to coagulation [130]. Erythrocytes can promote platelet aggregation and activate platelets, and activated platelets can promote erythrocyte aggregation [131]. Hemoglobin has been shown to enhance fibrinolysis. Therefore, erythrocytes play an important role in the fracture healing process.

1.2.3.1.3 Leukocytes: Monocytes and Macrophages

Leukocytes are composed of granulocytes, monocytes, and lymphocytes, which play different functions in immune defense. Leukocytes play an important part in anti-inflammation and sterilization. When the body is damaged, neutrophils are the first cells to migrate to the damaged tissue. Due to the short service life of neutrophils, the number decreases after 24–48 hours, and they are replaced by monocytes. Monocytes at the damaged site differentiate into long-lived macrophages under cytokines,

extracellular metabolites and hypoxia. Leukocytes are composed of granulocytes, monocytes and lymphocytes, which play different functions in immune defense. Leukocytes play an important role in anti inflammation and sterilization. When the body is damaged, neutrophils migrate to the damaged tissue for the first time. Due to the short service life of neutrophils, the number decreases after 24–48 hours and is replaced by monocytes. Monocytes at the damaged site differentiate into long-lived macrophages under cytokines, extracellular metabolites and hypoxia. Macrophages can be divided into two types: resident macrophages and inflammatory macrophages derived from circulating monocytes. Inflammatory macrophages can be divided into two types: M1 and M2. M1 type cells mainly serve an anti-inflammatory function. In contrast, M2 cells mainly function in tissue repair and angiogenesis in response to foreign injury. The resident macrophages in bone tissue, called bone macrophages (osteomacs), further show macrophage repair function during fracture healing. It was found that the distribution of macrophage subtypes was very important for the formation of intrachondral callus. Using the femoral fracture model, it was found that inflammatory macrophages existed in granulation tissue at the front of soft callus, while resident macrophages (osteomacs) existed in mature hard callus. In all, The above demonstrate that osteosarcoma (OS) is vital to the healing process during the formation of endochondral and intramembrane callus during bone fracture. Immune cells and osteoblasts interact through signaling of cytokines, signaling molecules, transcription factors, and receptors [132].

1.2.3.1.4 Mesenchymal Stem Cells (MSCs)

BMSCs can differentiate into a variety of cells, which include adipocytes, chondrocytes, and osteoblasts. During the inflammatory phase of fracture hematoma formation, various cytokines such as interleukin 1, interleukin 6, and tumor necrosis factor-alpha are released in large amounts and converge to the injury site, thereby activating the differentiation of BMSCs into a variety of cells [54]. In addition, platelet-derived growth factor and tumor necrosis factor-beta have been reported to stimulate the migration activation and proliferation of bone marrow MSCs [133]. There are various differences in the processes of intramembranous and endochondral ossification with different BMSC sources and their differentiation processes. In intramembranous ossification, BMSCs mainly originate from the periosteum and surrounding soft tissues and begin to proliferate and differentiate toward the formation of woven bone within 24 hours of fracture. In intrachondral ossification, BMSCs mainly originate from the bone marrow and bone cortex, and proliferate toward the cartilage scab within 72 hours of fracture [134]. Moreover, BMSCs play an important role in promoting angiogenesis during fracture healing. BMSCs highly express BMPs, which play an important role in the angiogenesis process.

1.2.3.2 Molecular Components in Bone Microenvironment

1.2.3.2.1 Transforming Growth Factor-β (TGF-β)

Activated platelets can produce transforming growth factor-β at the initial injury site, which participates in callus formation. Osteoblasts and chondrocytes can also produce TGF-β which can also promote the transformation of MSCs into osteoblasts

and chondrocytes. TGF-β plays an important role in cartilage formation and endochondral ossification. Moreover, TGF-β can induce the expression of a series of matrix molecules. TGF-β can induce chemotaxis of inflammatory cells to establish a positive feedback circuit of growth factors in damaged bones [135]. Transforming growth factor has the ability to recruit immune cells and induce fibrous matrix, which has been seen as an inflammatory factor [136]. TGF-β2 and TGF-β3 are assumed to make a difference in fracture healing because their expression peaks during cartilage formation, while the expression of TGF-β1 was relatively stable during the entire healing process.

1.2.3.2.2 Insulin-like Growth Factors (IGFs)

IGF-I (or somatomedin-C) and IGF-II (or bone growth factor) are growth factors with a similar structure to insulin. IGF-I is known to promote mitosis of preosteoblasts and osteoblasts. It was found that IGF-1 promotes osteogenesis of osteoblasts and enhances the action of PDGF during bone formation, thereby improving the overall healing rate [127]. Studies have suggested that IGF-I in articular cartilage is required for chondrocyte homeostasis, proteoglycan synthesis, and chondrocyte degradation. It has also been shown that IGF-I affects the cartilage differentiation of MSC independent of transforming growth factor [137]. This shows that IGF-I play a great role in cartilage formation.

1.2.3.2.3 Vascular Endothelial Growth Factor (VEGF)

A fracture hematoma is a hypoxic environment, and hypoxia stimulates macrophages to produce VEGF, which promotes angiogenesis. Leukocytes in the inflammatory phase of fracture hematoma can also release VEGF and PDGF to promote fracture healing [138]. Hypoxia inducible factors (HIFs) are transcriptionally active proteins that are stably present in response to hypoxia. The combination of HIFs and VEGF can regulate angiogenesis and bone formation under hypoxia. The activation of HIF-1 in osteoblast can increase the level of VEGF in osteoblasts and significantly increase angiogenesis and bone formation. Bone reconstruction involves two processes: bone resorption and bone formation. Osteoclasts adhering to the bone surface can resorb necrotic and damaged bone, while bone resorption recruits a large number of osteoblasts, which can secrete a large amount of bone matrix and then mineralize bone. In the balance between bone resorption and bone formation, the balance between the number and expression of osteoclasts and osteoblasts plays an important role. Vascular invasion of bone tissue plays an important role in bone homeostasis, as blood vessels bring nutrients and various cytokines necessary for bone remodeling. The vascular network of bone has a longitudinal Haver's pavilion and a transverse Harvard's canal forming the periosteal cortical vascular network, which facilitates the exchange of nutrients between blood vessels. The vasculature plays an important role in distraction osteogenesis and also prevents the process of distraction osteogenesis when the formation of blood vessels is inhibited [139]. These findings suggest that bone remodeling can be regulated by the molecular the local environment of bone.

1.2.4 Bone Microenvironment in Osteosarcoma (OS)

OS is a malignant tumor that occurs in adolescents, often in the epiphysis of long bones, such as the proximal tibia and distal femur. The bone microenvironment provides a suitable environment for tumor cell proliferation and migration, and provides sufficient conditions for the metastasis of tumors to be important [140]. The main components of bone microenvironment, such as hypoxia, acidosis, and chemokines, are crucial in the proliferation and metastasis of OS.

1.2.4.1 Mesenchymal Stem Cells (MSCs) and OS Metastasis

MSCs are considered to be the vital factor in the bone microenvironment to induce OS metastasis. OS comes from mesenchymal stem cells, and its development, metastasis, and drug resistance are highly related to mesenchymal stem cells. Mutations of tumor suppressor genes TP53 and Rb, aneuploidy of p16/CDKN2A, and genomic loss often lead to the transformation of MSCs into OS cells [141]. In a rat OS model, subcutaneous injection of rat OS cos1NR cells for three and five weeks followed by intravenous MSCs significantly promoted lung metastasis, but it did not affect tumor growth [142]. At the same time, gene expression analysis showed that compared to cos1NR cells, adhesion plaque, cytokine receptors and extracellular matrix receptor pathways were significantly altered. Cellular signaling pathway molecules are also altered during tumor metastasis and tumor angiogenesis. It was found that BMSCs and OS can interact with each other, and tumor cells can influence the bone microenvironment, which can likewise influence the proliferation and migration of tumor cells [143]. The study of tumor extracellular vesicles also confirmed the interaction between MSCs and OS. TGF carried in vesicles can stimulate MSC to release IL-6 expression [144]. The oxidative stress environment in the bone microenvironment of OS can stimulate lactate production in MSC. The acidic environment allows bone marrow MSCs to secrete a variety of cytokines, thereby promoting tumor metastasis [145].

1.2.4.2 Effect of Hypoxia Environment on OS Metastasis

Bone microenvironment is a hypoxic and acidic environment, which provides the necessary conditions for tumor metastasis. Hypoxia can stimulate the expression of HIF; therefore, many studies on HIF have been recently conducted. HIF1 overexpression promoted the invasion and proliferation of MG63 and U2OS cells, while Mir-20b and Mir-33b inhibited HIF1 expression and thus slowed down the proliferation of tumor cells. In the process of tumor metastasis, Lncrna malat1 could induce angiogenesis to promote tumor metastasis [146]. In the hypoxic bone microenvironment in OS, increased HIF1 content could regulate the expression of Angptl2 and subsequently promote bone tumor invasion and metastasis, in addition, it could improve the formation of osteoclasts and bone resorption [147].

1.2.4.3 Extracellular Vesicles (EVs) in the Tumor Microenvironment

Tumor cells can communicate between cells through extracellular vesicles, which play an important role in tumorigenesis, progression, metastasis, and invasion.

MSc-derived EV promotes OS growth by activating the hedgehog and PI3K/Akt signaling pathways. Meanwhile, OS-derived EV regulates the transformation of BMSCs by regulating LINE-1 hypermethylation in these cells [148]. OS-derived EV regulates BMSC transformation by modulating LINE-1 hypermethylation in these cells [149]. In addition, OS-derived EVs alter bone microenvironment remodeling by affecting gene expression [150].

1.3 Biomaterials and Bone Microenvironment

Bone microenvironment is easily affected by the following two factors: external environment and internal environment, which is the main cause of bone diseases [151]. Biomaterials play an indispensable role in restoring bone microenvironment. The interaction between cells and biomaterials is vital to affect the changes in bone microenvironment, including cell proliferation, differentiation, and regulation related factors [152]. At present, many biomaterials have been found to be used for the restoration of bone microenvironment. Their biocompatibility or biodegradability of biomaterials has great application value [153]. The biomaterials used in bone microenvironment are classified as follows according to different functions.

1.3.1 Biomaterials and Bone Cells

Bone cells usually differentiate into several types: BMSCs, osteocytes, osteoblasts (OB), osteoclasts (OC), and their precursors [154, 155]. BMSCs are a kind of cell subpopulations found in mammalian bone marrow stroma with multiple differentiation potentials to differentiate into bone, cartilage, fat, nerve, and myoblasts [156]. Compared to other cells, OB account for a large proportion in mature bone tissue [157]. OB is generated from bone, periosteum, bone marrow, and extra-osseous tissue; human embryonic skull and neonatal animal skull are common sources of osteoblasts [158]. A variety of biologically active substances are secreted by OB to regulate and influence the process of bone growing and reconstruction [159]. OB are also responsible for the synthesis, secretion, and mineralization of bone matrix [160]. Bone tissues are continuously reconstructed in the entire life process. Bone reconstruction is a complex process, which includes absorption and reformation of bone [160, 161]. OC, derived from the blood mononuclear-macrophage system, are a special terminally differentiated cell. It can form huge multinucleated cells by fusion of its mononuclear precursor cells in a variety of ways [162]. Osteoblasts and osteoclasts have different sources and functions and play important roles in bone reconstruction and bone disorder [163].

The abnormal state of these cells is interfered by different factors, which directly affects the changes in the bone microenvironment, but it can often be adjusted by the adjustment of biological materials [164, 165]. The stimulation of bone cell attachment, proliferation, and differentiation depends in part on the physical and chemical properties of the biological material such as the surface properties, chemical composition, static electricity, geometric structure, texture, and roughness [166].

The interaction between biomaterials and bone cells occurs mostly in bone reconstruction [167]. Most of the current research on cell-biomaterial interaction is conducted on osteoblasts; the materials used for bone repair included inorganic materials, natural polymers, synthetic polymers and even composite materials for bone replacement [168]. In addition, compared to bone, the main properties of bone substitutes, used for bone repairing, are their mechanical properties (elastic modulus, fatigue, and permeability) [168, 169]. Bone conductive inorganic materials are hydroxyapatite (HA), tricalcium phosphate (TCP), calcium phosphate bone cement, apatite wollastonite, and bioactive glass [170]. For example, the calcium phosphorus ratio of organic bone matrix is about 1.66, that of hydroxyapatite is about 1.67, and that of TCP is about 1.5 [171]. HA has chemometrics similar to minerals [171]. In clinical applications, calcium phosphate ceramics are usually used due to body tolerance, osteoconductivity, and bionic properties [172, 173]. In addition, chemical bonding with bones is used during implantation. Due to its deformation resistance, titanium (Ti) is also used in bone repair and hip prosthesis [174]. BMSCs attached to titanium fiber mesh can differentiate into osteoblasts *in vitro*, but some *in vivo* experiments emphasize the importance of using protein or HA coating to improve the osteogenic properties of titanium fiber mesh [175]. For example, Vlacic-Zischke et al. reported that micro-roughness was formed on the surface of Ti substrate by sand blasting and acid etching. The results showed that the modified surface increased the level of TGF- β signal transduction and stimulated osteoblast differentiation. Nanoscale surface morphology is also important for cell [176]. Lim et al. used PLA polystyrene films with a depth of 14~45 nm, and discovered that surface with shallower nano-dimple material results in more spreading and better adhesion of human fetal osteoblasts as compared to flat PLA surfaces [177]. The surface morphology of the materials will yet affect adsorption and integrins of protein of the cell surface, thereby regulating the behavior of materials for cell adhesion [178]. Cell proliferation, differentiation, and activity can also be regulated by the surface morphology of biomaterials. Some behaviors of cell can be affected by adjustment surface microstructure. Studies have found that the expression of osteoblast genes is intervened by titanium with different morphologies [179]. Compared to smooth surface (Ra = 0.6 μm), when they were on a sandblasted titanium surface (Ra = 4 μm), the expression of about 10% of the genes changed more than three times [180]. In addition, the micro-roughness degree of titanium surface not only affects the production of cytokines but also affects the angiogenesis and the function of BMSC [181].

Natural polymers and synthetic polymers form polymer materials for clinical use togethers. Natural polymers include polysaccharides (hyaluronic acid, alginate, collagen, etc.) or proteins (collagen, fibrin) [182]. For example, F. Munarin et al. reported that Pectin, derived from plant cell walls, provides properties as artificial ECM. The results revealed that pectin showed the potential to maintain the survival and differentiation of immobilized cells in the experiment of metabolic activity, morphology, and osteogenic differentiation of pre-immobilized MC3T3-E1 osteoblasts [183]. In the study of Elaine Quinlan et al., osteoblasts used in collagen hydroxyapatite (CHA) scaffolds were tested *in vitro*, which showed an enhanced pro-osteogenic effect [184].

Although these polymers have bone conduction properties, their mechanical properties are poor and difficult to be used in clinic. The following synthetic polymers can be used in clinical applications: polyethylene glycol, poloxamer, polyalpha-hydroxy acid, polyorthoester, polyanhydride, polyphosphazene, and polyphosphonate [185]. Polymer degradation can trigger an inflammatory response and affect cell adhesion and proliferation. At present, synthetic polymers have been widely studied, because they can combine the advantages of many single materials, and they can significantly overcome many shortcomings of a single material. Polymer-to-cell behavior is also affected by its chemical composition, molecular weight, and crystallinity. Seher Ozkan et al. developed a porous scaffold (discrete and continuous) composed of polycaprolactone (PCL), b-tricalcium phosphate (b-TCP) nanoparticles and salt porogen Gradient radial grading [186]. A scaffold with interconnected porosity, pore size distribution, and b-TCP nanoparticle can be obtained through this strategy. The compression properties of human fetal osteoblasts and cell proliferation *in vitro* show that this scaffold has a good application prospect.

Composite biomaterials, which contain a free combination of synthetic and inorganic materials, are particularly useful for preparing tissue engineering materials, which process sufficient bionic and mechanical properties [187]. Composite materials can also simulate the characteristics and morphology of cortical bone and trabecular bone [188]. In addition, the physical properties of biomaterials also affect the physiological changes of bone cells. Empirical observations of medical implants show that when using implants with high surface roughness, the interaction between bone and implant will be better [189]. Recent studies have proved that biomaterials with terminal polyethylene glycol (PEG), OH, COOH, NH_2, SH, and CH_3 can be used to evaluate the role of surface chemistry on the level of cell [190]. For example, alkyl mercaptans at the end of self-assembled monolayers affect the adhesion and function of osteoblasts [191]. The positive and negative of the surface charge of biological materials participate in the influence of cell behavior [166]. Rat skull osteoblasts are cultured on positively charged and negatively charged polymers, and their morphology is completely different [192]. Osteoblasts attach and diffuse more on positively charged hydrogels than on neutral or negatively charged hydrogels [193]. In recent years, short-chain peptides have been successfully immobilized on the surface of biomaterials by covalent attachment [194]. The addition of commonly used sequences like RGDs, consisted of arginine, glycine and aspartate, to biomaterials greatly aids in the attachment, growth and differentiation of osteoblast precursor cells [195]. Bone sialoprotein (FHRRIKA) sequence and fibronectin (PRRARV) peptide exhibit increased osteoblast and macrophage numbers specialty [196]. In the past 10 years, the research on bone tissue in three-dimensional polymer scaffolds has attracted much attention. One of the biggest disadvantages of a lot of stents is the restricted transportation of nutrients, oxygen, and waste removal [197]. Therefore, cells are only colonized on the surface of the scaffold because they are prone to necrosis in deeper parts. For example, R. DI LIDDO et al. reported that poly-ε-caprolactone scaffolds prepared with alginate threads (PCL-AT) with a pore size of 10–100 μm encapsulate HA and bone extracellular matrix (BEM). The results showed that the porosity grades of PCL-AT-HA and PCL-AT-BEM promote the best conditions for the growth

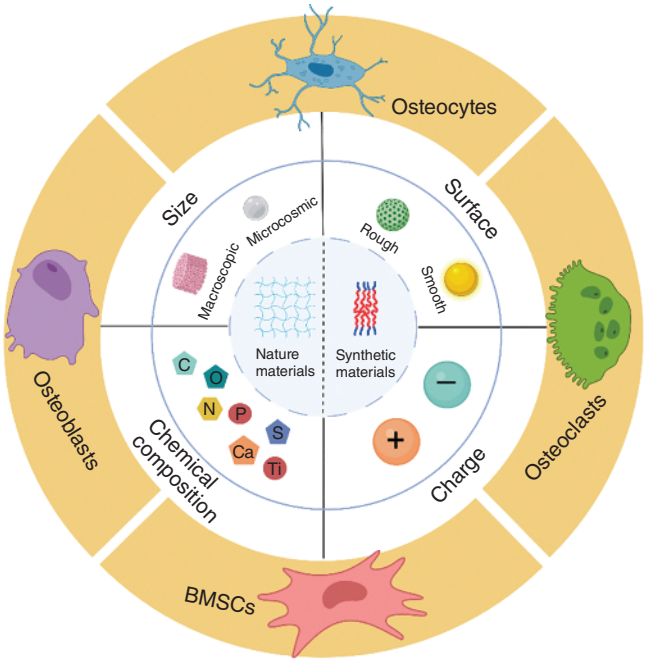

Figure 1.4 Effects of physical and chemical properties of biomaterials on bone cells.

of bone marrow-derived MSCs in the early stage [198]. Therefore, the ideal 3D bone graft scaffold should have a high specific surface area to achieve cell attachment and nutrient exchange, not just through diffusion.

In short, the physical and chemical properties of biological materials determine the changes in cell behavior, which is often an important indicator for evaluating the good biocompatibility of biological materials. The materials that affect the behavior of bone cells have been developed more widely and diversely. It can be found that by adjusting the physical and chemical properties of the materials (the difference in surface structure, functional groups, and the positive and negative charges), biomaterials with good cell compatibility can be optimized. However, this must be verified by *in vivo* experiments to prove the biocompatibility and functionality of the material (Figure 1.4).

1.3.2 Biomaterials and Bone Hematopoietic System

Bone marrow (BM) is the largest hematopoietic organ, is also the main place of hematopoiesis and an important repository of minerals [199]. Bone marrow is a kind of spongy tissue that exists in the mesh between the bone marrow cavity of long bone (such as humerus and femur) and the loose bone of flat bone (such as iliac bone) [200]. BM is divided into red bone marrow and yellow bone marrow. HSCs in red bone marrow have hematopoietic function. It can differentiate and develop into red blood cells, platelets, lymphocytes, granulocytes, etc. [201].

HSCs play an important role in stem cell transplantation in patients with blood diseases [202]. The important factor of its regenerative potential is the BM microenvironment [202]. In recent years, it has been found that changes in HSCs in the bone marrow microenvironment can often be adjusted with biological materials [203]. As mentioned earlier, bone biomaterials play an important role in bone microenvironment by providing matrix for cell adhesion, proliferation and differentiation, and by regulating cell activity and function. Another concern is that the directional differentiation of stem cells is induced for artificial biotransformation through the interaction between bone biomaterials and stem cells. Novel bone biomaterials have emerged, including biodegradable bioactive ceramics, polymers, and metals with good biocompatibility [204].

Studies have shown that the different chemical composition, surface characteristics, and morphology of bone biomaterials may promote the proliferation of HSCs *in vitro*, so that HSCs can differentiate into mature blood cells or serve as a drug testing platform [205]. For instance, A.C. Wilkinson, et al. found that polyvinyl alcohol (PVA) was identified as a substitute for serum albumin in culturing HSCs. Under the action of PVA, 100 ng/ml Thrombopoietin and 10 ng/ml stem cell factor, mouse CD34, LSK, and HSC were amplified *in vitro* and maintained function-activity [206]. Similarly, for $CD34^+$ hematopoietic stem and progenitor cell (HSPCs) derived from human cord blood, PVA can replace serum albumin [207]. However, compared to mouse HSCs, human $CD34^+$, $CD38^-$, $CD90^+$, $CD49f^+$, and HSCs are not sensitive to the hydrolysis state of PVA. Sambit Sahoo et al. developed a bio-hybrid fiber scaffold system by coating bioactive bFGF-releasing ultra-fine PLGA fibers on a mechanically strong, slow-degrading degummed knitted microfiber wire scaffold, which stimulated the proliferation of mesenchymal progenitor cells (MPCs) [208].

The introduction of biomolecules on the surface of biomaterials is the most commonly used biofunctionalization technology for cell culture [209]. Nanofibers (NFs) have been widely used in the past 20 years, due to their ability to mimic the ECM structure of many body tissues (such as bone marrow) [210]. To overcome the disadvantages of poor mechanical properties and poor processability of natural nanofibers, chemical surface treatment was performed on the surface of polymer NFS, and functional groups were introduced on the surface. K.-N. Chua, C.et al. reported that human CD34+ HSPCs cultured on grids of aminated polyethersulfone NFs exhibited stronger adhesion and larger HSPCs progenitor cell expansion and maintenance capacity than HSPCs cultured on unmodified, hydroxylated or carboxylated NFs grids or aminated membranes. This study shows that even simple surface chemistry can affect HSPC in the microenvironment [211].

The three-dimensional (3D) structure of biological materials can simulate microenvironment of bone hematopoietic system. Hydrogel is notable representative of biomaterials [212]. The properties of hydrogels can be adjusted according to the characteristics of the microenvironment, which makes them advantageous for certain applications or analytical methods [213]. In the field of *in vivo* and *in vitro* research based on biological materials, the use of hydrogel incorporation systems is extensive and diverse. The raw materials used to make 3D structured hydrogels can be natural ECM (such as fibrin, sodium alginate, chitosan, collagen, pullulan,

Table 1.1 Application of biomaterials in bone marrow hematopoietic system.

Biomaterials	Characteristic	Main functional group
Polyvinyl alcohol (PVA)	Replace serum albumin	−OH; C=C
PLGA microfiber wire scaffold	ECM-like biomimetic architecture	−COOH; −OH
Polyethersulfone nanofibers	Adhesion	$-SO^{2-}$
Natural hydrogels (fibrin, chitosan, collagen) Artificial hydrogel (polyethylene glycol, polyurethane)	3D structure; supporting paracrine (large pores) or autocrine (small pores) signaling	−OH; −COOH; $-NH_2$

cellulose, silk fibroin, etc.) or synthetic polymers (such as PEG, polyurethane, poly(lactic-*co*-glycolic acid), and PCL) [214]. However, when preparing hydrogels, natural sources and synthetic compounds are often used to prepare hydrogels together, which have improved physical and chemical properties. The research team of A.E. Gilchrist, S. successfully fabricated hydrogels with different pore sizes, supporting paracrine (large pores) or autocrine (small pores) signaling, and obtained cell behavior data. The results showed that when cultured alone, murine BM-derived lineage$^-$ Sca1$^+$ c-Kit$^+$ (LSK) HSPCs proliferated significantly in the paracrine signal supporting gelma matrix, but when HSPC and MSCs were co-cultured in the autocrine signal support gel, the expansion rate was much higher [215]. RUWAN D.et al. also described the successful cultivation of HMSCs in a three-dimensional collagen matrix under mechanical strain. In addition to providing a 3D structure, the hydrogels were also allowed to be tuned to have functionalization, mechanical properties, and degradability to improve compatibility with the human environment [216]. T. Bai et al. reported that a zwitterionic hydrogel with peptide chain cleavage sites was used to amplify HSPC derived from human cord blood and bone marrow and cultured for several generations. In immunocompromised mice, the number of long-term HSCs can be increased by 73 times and can be reconstituted for at least 24 weeks [205] (Table 1.1).

Part of the application of the above-mentioned materials for BM microenvironment is at the cellular level, and we are still facing great challenges. Often the application of biomaterials *in vitro* and *in vivo* will make a big difference, due to the complex and changeable environment in the body that is difficult to control.

1.3.3 Biomaterials and Bone Immune System

In addition to the main site of hematopoiesis, BM also contains lymphoid progenitor cells and mature immune cells (B cells, neutrophils, macrophages, and T cells) [217]. The immune system has the functions of immune surveillance, defense, and regulation. Immune cells exist in the bone microenvironment and interact with

bone to perform the functions of the "bone immune system" in concert [218]. The key role of the bone immune system in bone microenvironment against foreign bodies and pathogens has long been familiar to researchers [219]. With the clear understanding of this new research field of bone immunology, the reciprocal regulation between immune cells and bone morphogenetic cells has been studied in greater depth, and the two systems are thought to be tightly linked through various cytokines, signaling molecules, transcription factors, and receptors [164].

In the clinical treatment of bone defects, biomaterials show a significant therapeutic role, when bone microenvironment changes [220]. However, the host immune response determines the fate of implants *in vivo*, whether they are formed in new bone, wrapped in fibrous tissue, or used for drug delivery for decrease of autoimmune response in the bone microenvironment [221]. The traditional biomaterial design includes the manufacture of inert biomaterials that can stimulate osteogenesis; however, *in vivo* and *in vitro* often fail to achieve consistent evaluation results. This has led to the evolution of biomaterials for implants with bone immunomodulatory properties [222]. These orthopedic biomaterials are endowed with good bone immunomodulatory properties that can trigger the desired immune response for proper bone regeneration process [176]. Under these circumstances, to regulate the crosstalk with immune cells (macrophages, neutrophils), various methods have been adopted such as changing chemical/structural characteristics or adding biologically active molecules.

An ideal biomaterial should be able to stimulate good crosstalk between immune cells and cells of the skeletal system at different stages of bone healing. In this case, to be able to design biological materials that control the polarization of macrophages and the positive crosstalk with bone-forming cells, it is often through changing the chemical/topographic characteristics or adding biologically active molecules [223]. As mentioned earlier, the interaction between the surface of the biomaterial and the protein adsorption layer is critical for the emergence of an immune response to the implantable biomaterial. In this regard, existing studies confirmed that changing different surface chemical properties, hydrophilicity, surface charge or functional groups, can affect the response of immune cells [164]. Hydrophobicity or hydrophilicity of biomaterials is the key factor affecting protein adsorption. The hydrophilicity of biomaterials has a non-negligible relationship with protein layer adsorption, fibrin formation and clot formation [224]. Strongly hydrophilic biomaterials have inherent immunogenicity. Kakizawa et al. prepared monodisperse silica nanoparticles that showed different hydrophobic poly (amino acid) surface modification and reported that the secretion of IL-1 β and IFN- γ is related to the hydrophobicity of poly(amino acids) [225]. In addition, their research also showed that strong hydrophilic biomaterials can promote the process of bone regeneration. Li et al. discovered lower hydrophilicity of Ti surfaces can induce the secretion of a variety of pro-inflammatory cytokines (TNF) compared to heparin/fibronectin functionalized titanium surface-α, MCP-1, and IL-1β) [226]. The addition of hydrophilic molecules such as PEG and polyoxyethylene (PEO) to carriers and tissue engineering structures as surface modifiers for implants to improve their hydrophilicity and reduce protein adsorption [227]. Future strategies can use

changes in surface chemistry to regulate immune response to achieve natural healing response to injury. Immune response is also closely related to the surface charge of implanted biomaterials [228]. Therefore, the following functional groups, such as amino ($-NH_2$), hydroxyl ($-OH$), carboxyl ($-COOH$), are usually investigated, and *in vivo* applications have found that the amino and hydroxyl groups can induce immune cell infiltration and form a complex surrounding the thick fibrous capsule of the implant [229].

In the process of natural degradation in the microenvironment when biodegradable biomaterials are implanted, the immune response will also be affected by surface changes and degradation products [230]. Furthermore, there are great differences in the structure of biomaterials, and some of them can further promote the process during initial degradation, leading to structural collapse and loss of original functions. After the bio-implantation, the blood in the injured blood vessel begins to interact rapidly with the biomaterial. The surface properties of biomaterials can exhibit differences in the amount and type of adsorbed proteins and further recruitment and adhesion of various cells. Implantable biomaterials are not only passive targets when confronted with the host immune system, but they also have a dramatic effect: the magnitude and type of the implant-mediated immune response can be modulated.

In addition to surface chemistry, the morphological features and porosity of biomaterials also affect the plasticity and function of immune cells [231]. The surface roughness of biological materials can also affect the interaction with immune cells, which is a characteristic of biological materials. In the study of Ali K. Refai et al. on the effect of titanium (Ti) surface morphology on the activation of macrophages and the secretion of pro-inflammatory cytokines and chemokines, four topography were used: topography produced by mechanical polishing, coarse sandblasting, acid etching, sandblasting, and acid etching (SLA) [232]. It was found that unstimulated macrophages increased their pro-inflammatory cytokine (TNF-α) secretion when adhered to rough surfaces. This *in vitro* study showed that surface morphology, especially SLA surface, regulates the expression of macrophage pro-inflammatory cytokines and chemokines in a time-dependent manner. Generally speaking, the roughness can be presented on a micro-scale, and there is evidence that the micropatterned surface show a beneficial effect on the bone immune microenvironment, thereby increasing the success rate of implantation. For example, Hotchkiss et al. discovered that micro-roughness-modified Ti surface promotes phenotypic transformation of M2 macrophages with increased IL-4 and IL-10 cytokine production, while smooth Ti matrix promotes M1 polarization [233].

In recent years, nano-scale biomaterials have been extensively studied, because the surface depth of bone tissue is about 32 nm [234]. Biomaterials that can be surface-regulated at the nanoscale can directly affect important processes such as cell adhesion and proliferation. Chen et al. prepared a plasma-polymerized allylamine surface to modulate immune cell responses by immobilizing gold nanoparticles of different sizes (16–68 nm) [176, 235]. From the results obtained, the scale of the nanotopography can significantly modulate the immune microenvironment by altering the gene expression profiles of inflammatory cytokines.

Table 1.2 Summary of biomaterials that do not affect the immune system in the bone microenvironment.

Biomaterials				
Physical and chemical properties	Hydrophilicity	Ethanol amine	Polyoxyethylene (PEO)	Polyethylene glycol (PEG)
	Surfaces	Polydioxanone	Gold nanoparticles	Plasma-polymerized allylamine
	scaffold pore size and porosity			

The porosity and pore size of biomaterials are considered to be another relevant surface feature in that because the penetration of oxygen and nutrients can affect the fate of macrophages [236]. The small pore size on the surface will destroy the diffusion of nutrients and oxygen, especially in the interior of the implant material, resulting in a local hypoxia microenvironment. The local hypoxic environment leads to the development of a local inflammatory response, leading to the formation of granulation tissue and complete blockage of the pores, creating a barrier between the surrounding bone cells and the implant. In addition, appropriate hypoxic environments can stimulate the release of angiogenic growth factors, which are local host tissues necessary for angiogenesis [237]. Therefore, biomaterials should exhibit suitable pore sizes to enable the creation of a moderately hypoxic environment, which not only prevents inflammation but also promotes angiogenesis. Garg et al. showed that increasing polydioxanone scaffold pore size and porosity enhanced M2 macrophage markers. The surface with larger pores down-regulates the production of iNOS compared with smaller pores, which promotes the transition to the M1 phenotype [238].

Based on these observations, the strategy of adjusting the immune response by adjusting the physical and chemical properties of biological materials can be considered a valuable method. These biological materials can be used in smart drug delivery carriers, and materials that are friendly to the immune system can be used in the treatment of bone diseases, which will also provide a sufficient reference for developing new drug delivery systems for bone-related diseases (Table 1.2).

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