



## Review Article

## Multi-functional osteoclasts in matrix-based tissue engineering bone

Yue-Qi Chen<sup>a,1</sup>, Wen-Hui Hu<sup>b,1</sup>, Zi-Cai Dong<sup>b</sup>, Shi-Wu Dong<sup>a,b,c,\*</sup><sup>a</sup> Department of Orthopedics, Southwest Hospital, Third Military Medical University (Army Medical University), Chongqing, 400038, China<sup>b</sup> Department of Biomedical Materials Science, Third Military Medical University (Army Medical University), Chongqing, 400038, China<sup>c</sup> State Key Laboratory of Trauma, Burns and Combined Injury, Third Military Medical University (Army Medical University), Chongqing, 400038, China

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## ABSTRACT

The repair of bone defects, especially for the large segment of bone defects, has always been an urgent problem in orthopedic clinic and attracted researchers' attention. Nowadays, the application of tissue engineering bone in the repair of bone defects has become the research hotspot. With the rapid development of tissue engineering, the novel and functional scaffold materials for bone repair have emerged. In this review, we have summarized the multi-functional roles of osteoclasts in bone remodeling. The development of matrix-based tissue engineering bone has laid a theoretical foundation for further investigation about the novel bone regeneration materials which could perform high bioactivity. From the point of view on preserving pre-osteoclasts and targeting mature osteoclasts, this review introduced the novel matrix-based tissue engineering bone based on osteoclasts in the field of bone tissue engineering, which provides a potential direction for the development of novel scaffold materials for the treatment of bone defects.

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## Introduction

Bone defects refer to the loss of partial bones, which is a common disease in clinical orthopedics, especially the bone defects of extremities. Bone with a small size of defects can repair itself, but the length of the defect more than 1.5 times of the transverse diameter of the defective bone will cause delayed healing of bone.<sup>1</sup> There are many elements which could lead to bone defects such as trauma, infection and tumour. Traditional bone defect treatment methods have shortcomings, such as long treatment cycle, high cost, uncontrollable curative effect and complications (infection and nonunion), which bring a great burden to patients.<sup>2,3</sup> At present, autogenous bone transplantation is the gold standard for the treatment of bone defects, but it has a series of problems, such as limited bone mass in donor areas, postoperative infection and bone defects.<sup>4</sup> In recent years, the rapid development of tissue engineering bone technology and materials has brought novel ideas and strategies for the treatment of bone defects.

According to the traditional theory, tissue engineering bone plays the role of osteogenesis directly through modulating seed cells.<sup>5</sup> Interestingly, the latest researches have put forward a novel conception of "bone remodeling in matrix microenvironment" and expanded the classical theory that the activated osteogenesis role of tissue engineering bone depends on seed cells directly. It has laid a theoretical foundation for the development of highly bioactive bone regenerative materials.<sup>6</sup> The critical factor of osteogenic effect of tissue engineering bone is the extracellular matrix secreted by seed cells. The extracellular matrix secreted by seed cells *in vitro* can promote the involvements of the host cells in the matrix microenvironment during bone regeneration, so as to provide significant osteogenic activities for scaffold.<sup>7</sup> This kind of scaffold material is constructed by removing the activity of seed cells in tissue engineering bone and retaining a variety of cytokines secreted by the cells. Then it is called "matrix-based tissue engineering bone (M-TEB)". It effectively overcomes the difficulties of traditional tissue engineered bones such as long production time, high cost, and unstable state of autologous stem cells in patients.<sup>8,9</sup>

Tissue engineering bone based on extracellular matrix performs a promising method for repairing bone defects, in which the seed cells are mainly mesenchymal stem cells (MSCs).<sup>10</sup> However, bone remodeling is a complex biological process of coupling among osteoclasts and osteoblasts.<sup>11,12</sup> Therefore, the important role of osteoclasts in M-TEB may be an effective strategy for bone

\* Corresponding author. Department of Orthopedics, Southwest Hospital, Third Military Medical University (Army Medical University), Chongqing, 400038, China.

E-mail address: [dongshiwu@tmmu.edu.cn](mailto:dongshiwu@tmmu.edu.cn) (S.-W. Dong).

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<sup>1</sup> These authors are equal to this work.

regeneration. It is well known that osteoclasts are monocyte/macrophage lineage cells differentiated from hematopoietic stem cells, and the particularity of their origin determines the multifaceted roles of osteoclasts in the skeletal system.<sup>13,14</sup> Different from bone-resorbing osteoclasts, the interactions among pre-osteoclasts (pOCs) with endothelial cells and MSCs are involved in angiogenesis and osteogenesis during the process of bone remodeling.<sup>15</sup> We are struggled to develop a suitable method to inhibit mature osteoclasts (mOCs) with effective bone resorption activity and preserve pOCs with positive osteogenic function. Therefore, from the point of view of preserving pOCs and targeting mOCs, this review introduces the new M-TEB based on osteoclasts in the field of bone tissue engineering, which provides a potential direction for the research of scaffold materials for the treatment of bone defects.

### A brief introduction of osteoclasts

Different from other bone cells, osteoclasts are derived from hematopoietic stem cells belonging to the monocyte/macrophage lineage. Receptor activator of NF- $\kappa$ B ligand (RANKL) and macrophage colony-stimulating factor (M-CSF) play important roles in all stages of osteoclasts differentiation.<sup>16</sup> mOCs are defined as multinucleated giant cells (more than 3 nuclei), and the process of differentiation and maturation can be described as follows. Monocytes proliferate massively under the stimulation of M-CSF and differentiate into mononuclear pOCs under the combined action of RANKL and M-CSF. Successively, pOCs fuse to form multinucleated osteoclasts under the continuous action of RANKL. Finally, over-activated osteoclasts perform high osteolytic efficiency but display a short lifespan.<sup>17</sup> Moreover, pOCs and mOCs are all derived from monocytes. In addition, they have shown the differences of the function. pOCs always play an essential role in the vasculargenesis during bone remodeling through secreting the cytokines such as platelet-derived growth factor-BB (PDGF-BB), TGF- $\beta$  and any others, wherever mOCs always make effects on bone erosion so as to initiate the bone matrix turnover.

The particularity of the origin of osteoclasts determines the multifaceted roles of osteoclasts in the skeletal system. In addition to their effective bone resorption activity, osteoclasts also play crucial roles in angiogenesis, osteoimmunology and hematopoietic niche formation.<sup>18–21</sup> The coupling among bone-resorbing osteoclasts and bone-forming osteoblasts acts as the basis of bone remodeling, which maintains the balance of calcium/phosphate ratio and bone homeostasis.<sup>22–24</sup> Abnormal osteoclast function can lead to various diseases. The most common primary type I osteoporosis (postmenopausal osteoporosis) is caused by the disappearance of the inhibitory effect of estrogen on osteoclasts in postmenopausal women, which underlies overactivated function of osteoclasts contributes to an increase in bone resorption and a decrease in bone mineral density.<sup>25–27</sup> The reduction of osteoclast bone erosion activity may result in some congenital diseases, such as bone dysplasia and osteopetrosis.<sup>28</sup> In addition, osteoclasts also play a critical role in bone metastasis of tumors. It is shown the malignant osteolysis is caused by overactivated osteoclastic bone resorption.<sup>29</sup>

### Interaction between osteoclasts and various cells in bone remodeling

In the process of bone remodeling, osteoclasts interact with various kinds of cells in the skeletal system, and the balance of these interactions finally completes bone repair. These cells involved in this process include osteoblasts, MSCs, endothelial cells, and so on.

### Interaction among osteoclasts and osteoblasts

The interaction between osteoclasts and osteoblasts has been studied most deeply, and they played essential roles in the process of bone remodeling and repair. The process of bone remodeling could be roughly divided into three stages: initiation phase, transition phase and termination phase.<sup>30</sup> In the initiation phase, hematopoietic progenitor cells are recruited, and osteoblasts secrete RANKL and M-CSF to promote their osteoclast differentiation and subsequent bone resorption. The transition phase is characterized by inactivation of bone resorption activity and a bias towards activation of bone formation activity through the crosstalk among osteoclasts and osteoblasts. Finally, bone remodeling is completed in the termination phase.<sup>31</sup>

Various cytokines play important roles in different stages during bone remodeling. In the initiation phase, osteoblasts recruit monocytes by secreting monocyte chemoattractant protein (MCP)-1.<sup>32</sup> In addition, CXC chemokine ligand 12 (CXCL12) is also considered to be as an important cytokine for recruiting osteoclast progenitor cells.<sup>33</sup> CXCL12 binds to its receptor CXC chemokine receptor 4 (CXCR4) on osteoclast progenitor cells and promotes the expression of osteoclastic resorption marker gene such as matrix metalloproteinase-9.<sup>34,35</sup> In the transition phase, the inhibition of osteoclasts may occur due to the apoptosis of osteoclasts. At this time, the apoptosis of osteoclast involves three pathways: Bim/caspase-3-dependent apoptosis,<sup>36</sup> estrogen-induced activation of Fas ligand<sup>37</sup> and increased extracellular calcium concentration due to the process of bone resorption.<sup>38</sup> The coupling relationship among osteoclasts and osteoblasts is carried out not only by cytokines, but also by direct communication. The most important pathway in direct contact is the interaction of ephrinB2/EphB4.<sup>39</sup> In the termination phase, the classical Wnt pathway activates the expression of osteoprotegerin through  $\beta$ -catenin, which further inhibits osteoclast differentiation and promotes osteogenesis.<sup>40</sup> In addition, osteoclasts express Notch1 and Notch3 receptors, which inhibit the function of osteoclasts through Notch signalling.<sup>41</sup>

### Interaction among osteoclasts and mesenchymal stem cells

MSCs have been proved to regulate the differentiation of macrophages, but the research on their roles in osteoclast differentiation is limited. Several studies have confirmed that MSCs inhibited the differentiation and maturation of osteoclasts in humans.<sup>42,43</sup> However, it has been reported that MSCs promoted the differentiation and maturation of osteoclasts by increasing the expression of RANKL and M-CSF in mice.<sup>44</sup> In the regulation of osteoclasts by MSCs, the role of CD200-CD200R axis is very important. MSCs inhibit the differentiation of osteoclasts through the expression of CD200 on the surface.<sup>42</sup> During bone remodeling, pOCs secrete PDGF-BB to recruit osteoprogenitor cells from the type H vessels to the bone remodeling site for the differentiation into bone-forming osteoblasts.<sup>45</sup>

### Interaction among osteoclasts and endothelial cells

The interaction among endothelial cells and osteoclasts regulates differentiation, maturation and bone-phagocytic ability of osteoclasts. In turn, osteoclasts modulate the angiogenic ability of endothelial cells. It has been reported that Netrin-4 secreted by endothelial cells inhibited the differentiation of osteoclasts and ameliorated bone loss *in vivo*.<sup>46</sup> However, vascular endothelial growth factor (VEGF)-C and VEGF-D produced by endothelial cells promote the differentiation of peripheral blood monocyte into osteoclasts.<sup>47</sup> Xie et al.<sup>48</sup> confirmed that PDGF-BB derived from immature non-resorbing pOCs binds to its receptor PDGFR $\beta$ , and

triggers the downstream cascades of phosphatidylinositol 3-kinase/protein kinase B and mitogen-activated protein kinases signal pathways to promote the migration and differentiation of bone marrow-derived endothelial progenitor cells and angiogenesis during bone remodeling in response to mechanical loading.

### M-TEB with osteoclasts

#### *A brief description of M-TEB*

The treatment of bone defects has always been one of the difficulties in orthopedic clinic. Autogenous bone transplantation serves as the gold standard of bone graft, but it belongs to invasive repair mode and the source of autogenous bone is limited. There are increasing cases of patients with bone defects per year.<sup>49</sup> However, at present, the development of bone graft materials which comply with the existing standards only meets 6% of the demand, causing a huge gap.<sup>50</sup> Fortunately, the therapeutic strategy based on tissue engineering technology for bone reconstruction has a broad application prospect. MSCs, which can differentiate into osteoblasts, plays a decisive role in bone repair in traditional tissue engineered bone. However, this kind of tissue engineering bone has the following disadvantages. Firstly, the autologous cells cannot be supplied on a large scale, and the process of preparation is complex. In addition, the living cell-dependent model leads to a valid period of only a few days, and harsh conditions of storage and transportation.<sup>10,51</sup> The concept of M-TEB has been put forward by our group in recent years. M-TEB can solve the bottleneck problems encountered in traditional tissue engineering bone, which was constructed by establishing the bank of stem cell derived from umbilical cord blood, removing seed cell activity and retaining a variety of cytokines secreted by these cells in tissue engineering bone.<sup>52</sup> In this construction technique, although the cell activity is removed, the cytokines and matrix proteins wrapped on the scaffold material by autocrine are still retained. After *in vivo* transplantation, the cytokines and proteins wrapped in the matrix under the action of enzymes are slowly released at the site of injury and participate in bone remodeling. Further chip detection has indicated that M-TEB was rich in recruitment factors (such as granulocyte chemotactic protein-2, macrophage inflammatory protein-3a, MCP-3 and urokinase-type plasminogen activator receptor), growth factors (such as insulin-like growth factor binding protein-3 and VEGF), and osteogenic factors (such as basic fibroblast growth factor, insulin-like growth factor-1 and osteoprotegerin).<sup>6,52</sup>

#### *The critical role of osteoclasts in M-TEB*

Osteoclasts are initially thought to be harmful to bone regeneration. With the gradual elucidation of the mechanism of osteoclast differentiation, the role of osteoclasts in bone regeneration is essential. PDGF-BB derived from tartrate resistant acid phosphatase-positive pOCs are capable of coordinating osteogenesis accompanied by angiogenesis during bone remodeling.<sup>53</sup> Based on this conclusion, accumulative studies have added osteoclasts as seed cells to the scaffolds of tissue engineering bone.<sup>54,55</sup> It has been reported that the lack of osteoclasts will lead to abnormal bone formation *in vitro*.<sup>56</sup> As we all know, the process of bone remodeling depends on the cooperation of a variety of cells in bone tissue.<sup>57</sup> Recent studies have shown that the coupling of osteoblasts and osteoclasts plays an important role in bone remodeling and regeneration. The extracellular matrix secreted by MSCs alone cannot simulate the complex microenvironment of bone repair.<sup>13</sup> With the refinement of 3D structure of scaffold materials and the improvement of 3D printing technology, the coculture of MSCs and osteoclasts as the seed system to construct tissue engineering bone

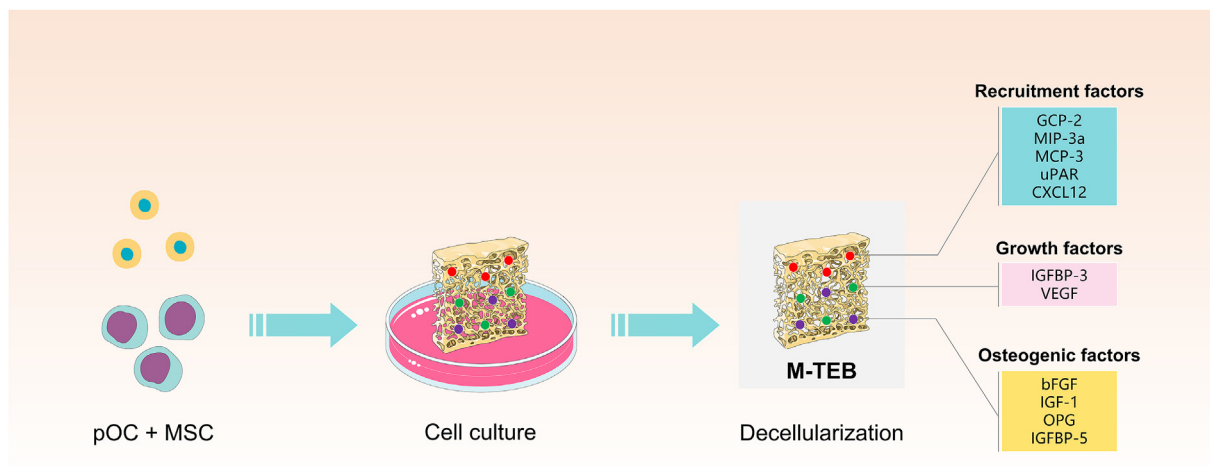
repair materials has been becoming a new research trend. Our group put forward the idea of adding pOCs as seed cells to construct M-TEB on the basis of MSCs. It has been found that 10:1 of MSC:pOC is the most suitable coculture ratio for bone repair and bone formation, and this coculture mode of compound seed cells secretes and accumulates more matrix protein than MSCs alone, which has better physiological function and bone repair potential in the rat model of femoral defect. Mechanistically, the isobaric tag for relative and absolute quantitation-labeled mass spectrometry showed that CXCL12 and insulin-like growth factor binding protein-5 proteins mainly released from pOCs promoted the migration and osteogenic differentiation of MSCs at the bone defect site in hosts, respectively. Therefore, pOCs were introduced into bone tissue engineering for the first time in our study, and an emerging bone regeneration material was constructed to treat bone defects by using the strategy of extracellular M-TEB (Fig. 1).<sup>8</sup>

#### *Regulating the function of osteoclast to realize "osteogenesis and angiogenesis" for integral bone repair*

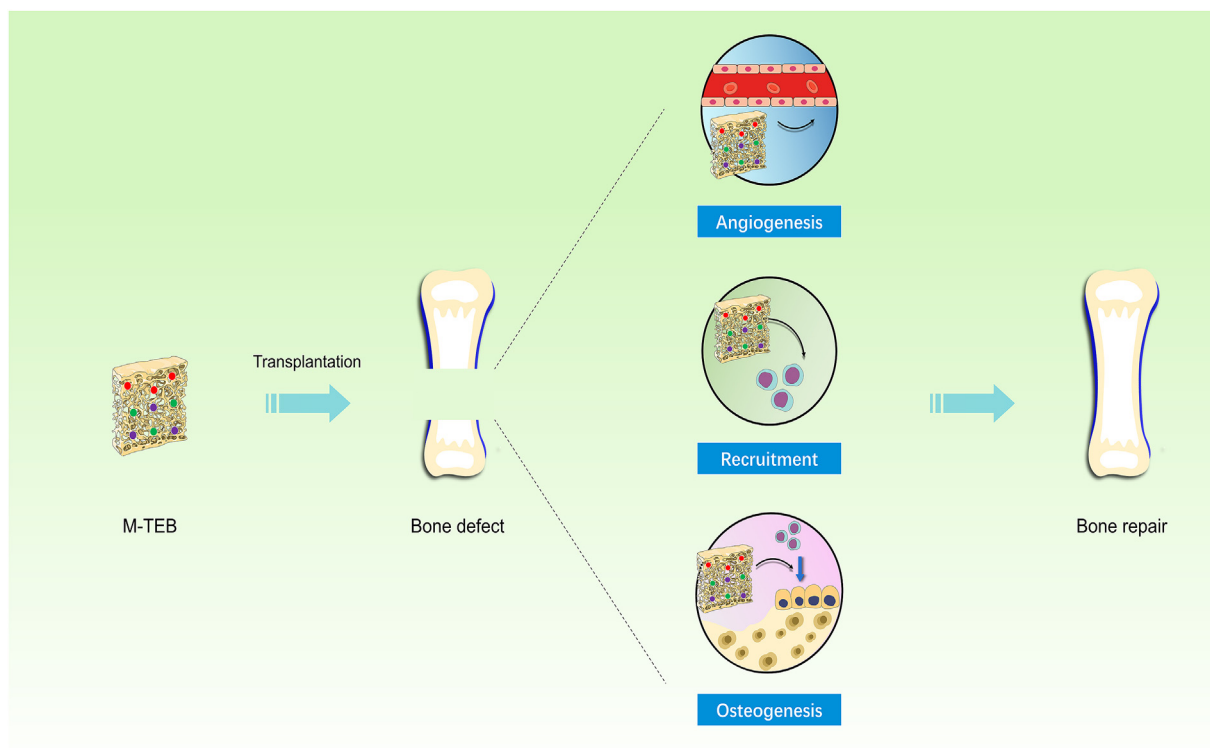
Accumulating studies have revealed the key role and mechanism of pOCs in bone repair and angiogenesis.<sup>12,58</sup> Therefore, effectively preserving pOCs to promote osteogenesis and angiogenesis and specifically targeting mOCs may be a novel strategy to promote bone regeneration and treat bone defects. The characteristics and modification of scaffolds will have different effects on the differentiation and bone resorption of osteoclasts. Studies have shown that the proportion of osteoclast differentiation and maturation increase with the roughness of materials.<sup>59</sup> In addition, it is worth noting that the difficulty of decalcification in the materials also affect the biological characteristics of osteoclasts. Tricalcium phosphate (TCP) is more easily absorbed by osteoclasts than hydroxyapatite to cause a high calcium concentration in osteoclast interface, thus inhibiting the differentiation of osteoclasts and promoting the apoptosis of osteoclasts.<sup>60</sup> A recent study has demonstrated that a scaffold loaded with Mn-TCP was designed to inhibit osteoclast formation and function by Mn<sup>2+</sup> release-dependent elimination of reactive oxygen species and activation of Nrf2 expression in osteoclasts (unpublished). In addition, the establishment of polyethylenimine functionalized graphene oxide complex loaded with miR-7b overexpression plasmid reduced the expression of osteoclast target protein dendritic cell-specific transmembrane protein, thus preventing the cell fusion of pOCs. Osteoclasts without dendritic cell-specific transmembrane protein cannot be fused into mOCs, and therefore bone regeneration is promoted by increased osteogenesis and angiogenesis by the production of PDGF-BB. All in all, bone repair scaffolds are modified to regulate osteoclast differentiation and function in order to achieve the effect of osteogenesis and angiogenesis for integral bone repair, which provides new perspectives on bone tissue engineering (Fig. 2).<sup>15</sup>

### Conclusion

Although the treatment techniques of bone defects are constantly improving, most of them cannot meet the demands of patients with bone defects. With the development of biomaterials and progress of medical technology, the emergence of tissue engineering bone has performed novel ideas for the treatment of bone defects. At present, the research direction of bone tissue engineering mainly refers to the selection of ideal seed cells, the technology of seed cell culture *in vitro*, the selection of scaffold materials and the solution of vascularization.<sup>61</sup> However, this review focuses on the M-TEB system and its application prospect in the repair of bone defects. The establishment of this theory breaks



**Fig. 1.** The synthetic route and procedure of matrix-based tissue engineering bone with preosteoclasts and MSCs.



**Fig. 2.** The transplantation of M-TEB dependent on preosteoclasts realize "osteogenesis and angiogenesis" for integral bone repair at the site of bone defects.

through the restriction of autologous construction mode and living cell dependent mode of traditional tissue engineering bone. Intriguingly, bioactive matrix proteins secreted by seed cells are the crucial factor and start-up switch for the osteogenesis effect of tissue engineering bone.

Osteoclasts, more than "bone eaters", play an important role in bone remodeling, angiogenesis, osteoimmunology and hematopoietic niche formation.<sup>14</sup> The repair of bone defects by M-TEB depends on a variety of bioactive matrix proteins (such as recruitment factors, growth factors and osteogenic factors) produced by MSCs.<sup>51</sup> However, bone remodeling is a complex biological process in which osteoclasts are coupled with multiple types of cells.<sup>11</sup>

Therefore, the important role of osteoclasts in M-TEB may be an effective strategy for bone regeneration. Finally, the modification of bone repair scaffolds effectively preserves the pOCs to promote vascularization and osteogenesis and specifically target to eliminate mOCs, which provides a potential direction for the research of scaffold materials for the treatment of bone defects.

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### Ethical statement

Not applicable.

### Declaration of competing interest

All authors declare that there are no conflicts of interest.

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All authors provided a substantial contribution to the conception, design and interpretation of the work, drafted the work or revised it critically for important intellectual content, and provided final approval of the submitted version of the manuscript.

### Author contributions

Yue-Qi Chen and Shi-Wu Dong contributed to the study design and conceptualization. Wen-Hui Hu and Zi-Cai Dong carried out data configuration. Yue-Qi Chen and Shi-Wu Dong wrote and prepared the original manuscript draft. Yue-Qi Chen, Wen-Hui Hu, Zi-Cai Dong and Shi-Wu Dong were responsible for reviewing and editing the final draft.

### References

- Yu Y, Ma L, Zhang H, et al. EPO could be regulated by HIF-1 and promote osteogenesis and accelerate bone repair. *Artif Cells Nanomed Biotechnol.* 2020;48:206–217.
- Buie HR, Bosma NA, Downey CM, et al. Micro-CT evaluation of bone defects: applications to osteolytic bone metastases, bone cysts, and fracture. *Med Eng Phys.* 2013 Nov;35:1645–1650.
- Hogan A, Heppert VG, Suda AJ. *Osteomyelitis.* *Arch Orthop Trauma Surg.* 2013 Sep;133:1183–1196.
- Chatterjea A, Meijer G, van Blitterswijk C, et al. Clinical application of human mesenchymal stromal cells for bone tissue engineering. *Stem Cell Int.* 2010;2010:215625.
- Perez JR, Kouroupis D, Li DJ, et al. Tissue engineering and cell-based therapies for fractures and bone defects. *Front Bioeng Biotechnol.* 2018 Jul 31;6:105.
- Deng M, Tan J, Hu C, et al. Modification of PLGA scaffold by MSC-derived extracellular matrix combats macrophage inflammation to initiate bone regeneration via TGF- $\beta$ -induced protein. *Adv Healthc Mater.* 2020 Jul;9, e2000353.
- Cunniffe GM, Diaz-Payno PJ, Sheehy EJ, et al. Tissue-specific extracellular matrix scaffolds for the regeneration of spatially complex musculoskeletal tissues. *Biomaterials.* 2019;188:63–73.
- Dong R, Bai Y, Dai J, et al. Engineered scaffolds based on mesenchymal stem cells/preosteoclasts extracellular matrix promote bone regeneration. *J Tissue Eng.* 2020 Jun 7;11, 2041731420926918.
- Marolt Presen D, Traweger A, Gimona M, et al. Mesenchymal stromal cell-based bone regeneration therapies: from cell transplantation and tissue engineering to therapeutic secretomes and extracellular vesicles. *Front Bioeng Biotechnol.* 2019 Nov 27;7:352.
- Hou T, Xu J, Wu X, et al. Umbilical cord Wharton's Jelly: a new potential cell source of mesenchymal stromal cells for bone tissue engineering. *Tissue Eng.* 2009 Sep;15:2325–2334.
- Hasegawa T, Tsuchiya E, Abe M, et al. Cellular interplay of bone cells and vascular endothelial cells in bone. *Clin Calcium.* 2016 May;26:677–682.
- Peng Y, Wu S, Li Y, Crane JL. Type H blood vessels in bone modeling and remodeling. *Theranostics.* 2020 Jan 1;10:426–436.
- Chen Y, Wang Y, Tang R, et al. Dendritic cells-derived interferon- $\lambda$ 1 ameliorated inflammatory bone destruction through inhibiting osteoclastogenesis. *Cell Death Dis.* 2020;11(6):414.
- Chen Y, Wang Y, Hu J, et al. Epithelone B prevents lipopolysaccharide-induced inflammatory osteolysis through suppressing osteoclastogenesis via STAT3 signaling pathway. *Aging (N Y).* 2020;12(12):11698–11716.
- Dou C, Ding N, Luo F, et al. Graphene-based microRNA transfection blocks preosteoclast fusion to increase bone formation and vascularization. *Adv Sci.* 2017 Dec 4;5, 1700578.
- Dong Y, Chen Y, Zhang L, et al. P2X7 receptor acts as an efficient drug target in regulating bone metabolism system. *Biomed Pharmacother.* 2020;125, 110010.
- Boyle WJ, Simonet WS, Lacey DL. Osteoclast differentiation and activation. *Nature.* 2003 May 15;423:337–342.
- Zupan J, Jeras M, Marc J. Osteoimmunology and the influence of pro-inflammatory cytokines on osteoclasts. *Biochem Med.* 2013;23:43–63.
- Feng W, Xia W, Ye Q, et al. Osteoclastogenesis and osteoimmunology. *Front Biosci.* 2014 Jan 1;19:758–767.
- Blin-Wakkach C, Rouleau M, Wakkach A. Roles of osteoclasts in the control of medullary hematopoietic niches. *Arch Biochem Biophys.* 2014 Nov 1;561:29–37.
- Zhu K, Jiao H, Li S, et al. ATF4 promotes bone angiogenesis by increasing VEGF expression and release in the bone environment. *J Bone Miner Res.* 2013 Sep;28:1870–1884.
- Ishii M, Kikuta J. Sphingosine-1-phosphate signaling controlling osteoclasts and bone homeostasis. *Biochim Biophys Acta.* 2013 Jan;1831:223–227.
- Abe T, Shin J, Hosur K, et al. Regulation of osteoclast homeostasis and inflammatory bone loss by MFG-E8. *J Immunol.* 2014 Aug 1;193:1383–1391.
- Fujita K, Iwasaki M, Ochi H, et al. Vitamin E decreases bone mass by stimulating osteoclast fusion. *Nat Med.* 2012 Mar 4;18:589–594.
- Faienza MF, Ventura A, Marzano F, et al. Postmenopausal osteoporosis: the role of immune system cells. *Clin Dev Immunol.* 2013;2013:575936.
- Buehring B, Viswanathan R, Binkley N, et al. Glucocorticoid-induced osteoporosis: an update on effects and management. *J Allergy Clin Immunol.* 2013 Nov;132:1019–1030.
- Rachner TD, Khosla S, Hofbauer LC. Osteoporosis: now and the future. *Lancet.* 2011 Apr 9;377:1276–1287.
- Sobacchi C, Schulz A, Coxon FP, et al. Osteopetrosis: genetics, treatment and new insights into osteoclast function. *Nat Rev Endocrinol.* 2013 Sep;9:522–536.
- Hu W, Zhang L, Dong Y, et al. Tumour dormancy in inflammatory microenvironment: a promising therapeutic strategy for cancer-related bone metastasis. *Cell Mol Life Sci.* 2020 Dec;77:5149–5169.
- Hadjidakis DJ, Androulakis II. Bone remodeling. *Ann N Y Acad Sci.* 2006 Dec;1092:385–396.
- Zhang L, Dong Y, Wang Y, et al. Sphingosine-1-phosphate (S1P) receptors: promising drug targets for treating bone-related diseases. *J Cell Mol Med.* 2020 Apr;24:4389–4401.
- Mulholland BS, Forwood MR, Morrison NA. Monocyte chemoattractant protein-1 (MCP-1/CCL2) drives activation of bone remodelling and skeletal metastasis. *Curr Osteoporos Rep.* 2019 Dec;176:538–547.
- Li X, Qin L, Bergenstock M, et al. Parathyroid hormone stimulates osteoblastic expression of MCP-1 to recruit and increase the fusion of pre/osteoclasts. *J Biol Chem.* 2007 Nov 9;282:33098–33106.
- Yu X, Huang Y, Collin-Osdoby P, et al. Stromal cell-derived factor-1 (SDF-1) recruits osteoclast precursors by inducing chemotaxis, matrix metalloproteinase-9 (MMP-9) activity, and collagen transmigration. *J Bone Miner Res.* 2003 Aug;18:1404–1418.
- Wright LM, Maloney W, Yu X, et al. Stromal cell-derived factor-1 binding to its chemokine receptor CXCR4 on precursor cells promotes the chemotactic recruitment, development and survival of human osteoclasts. *Bone.* 2005 May;36:840–853.
- Wakeyama H, Akiyama T, Takahashi K, et al. Negative feedback loop in the Bim-caspase-3 axis regulating apoptosis and activity of osteoclasts. *J Bone Miner Res.* 2007 Oct;22:1631–1639.
- Nakamura T, Imai Y, Matsumoto T, et al. Estrogen prevents bone loss via estrogen receptor alpha and induction of Fas ligand in osteoclasts. *Cell.* 2007 Sep 7;130:811–823.
- Zaidi M, Datta HK, Patchell A, et al. Calcium-activated' intracellular calcium elevation: a novel mechanism of osteoclast regulation. *Biochem Biophys Res Commun.* 1989 Sep 29;163:1461–1465.
- Zhao C, Irie N, Takada Y, et al. Bidirectional ephrinB2-EphB4 signaling controls bone homeostasis. *Cell Metabol.* 2006 Aug;4:111–121.
- Tatsumi S, Ishii K, Amizuka N, et al. Targeted ablation of osteocytes induces osteoporosis with defective mechanotransduction. *Cell Metabol.* 2007 Jun;5:464–475.
- Yu J, Canalis E. Notch and the regulation of osteoclast differentiation and function. *Bone.* 2020 Sep;138:115474.
- Varin A, Pontikoglou C, Labat E, et al. CD200R/CD200 inhibits osteoclastogenesis: new mechanism of osteoclast control by mesenchymal stem cells in human. *PLoS One.* 2013 Aug 5;8, e72831.
- Oshita K, Yamaoka K, Udagawa N, et al. Human mesenchymal stem cells inhibit osteoclastogenesis through osteoprotegerin production. *Arthritis Rheum.* 2011 Jun;63:1658–1667.
- Zhu H, Jiang XX, Guo ZK, et al. Tumor necrosis factor- $\alpha$  alters the modulatory effects of mesenchymal stem cells on osteoclast formation and function. *Stem Cell Dev.* 2009 Dec;18:1473–1484.
- Xu T, Luo Y, Kong F, et al. GIT1 is critical for formation of the CD31hiEmcnihi vessel subtype in coupling osteogenesis with angiogenesis via modulating preosteoclasts secretion of PDGF-BB. *Bone.* 2019 May;122:218–230.
- Enoki Y, Sato T, Tanaka S, et al. Netrin-4 derived from murine vascular endothelial cells inhibits osteoclast differentiation in vitro and prevents bone loss in vivo. *FEBS Lett.* 2014 Jun 27;588:2262–2269.
- Motokawa M, Tsuka N, Kaku M, et al. Effects of vascular endothelial growth factor-C and -D on osteoclast differentiation and function in human peripheral blood mononuclear cells. *Arch Oral Biol.* 2013 Jan;58:35–41.
- Xie H, Cui Z, Wang L, et al. PDGF-BB secreted by preosteoclasts induces angiogenesis during coupling with osteogenesis. *Nat Med.* 2014 Nov;20:1270–1278.

49. Gage J, Liporace A, Egol A, et al. Management of bone defects in orthopedic trauma. *Bull Hosp Jt Dis.* 2013;76:4–8, 2018 Mar.
50. Shakouri-Motlagh A, O'Connor AJ, Brennecke SP, et al. Native and solubilized decellularized extracellular matrix: a critical assessment of their potential for improving the expansion of mesenchymal stem cells. *Acta Biomater.* 2017 Jun;55:1–12.
51. Deng M, Chang Z, Hou T, et al. Sustained release of bioactive protein from a lyophilized tissue-engineered construct promotes the osteogenic potential of mesenchymal stem cells. *J Orthop Res.* 2016 Mar;34:386–394.
52. Deng M, Mei T, Hou T, et al. TGF $\beta$ 3 recruits endogenous mesenchymal stem cells to initiate bone regeneration. *Stem Cell Res Ther.* 2017 Nov 10;8:258.
53. Su W, Liu G, Liu X, et al. Angiogenesis stimulated by elevated PDGF-BB in subchondral bone contributes to osteoarthritis development. *JCI Insight.* 2020 Apr 23;5, e135446.
54. Detsch R, Schaefer S, Deisinger U, et al. In vitro: osteoclastic activity studies on surfaces of 3D printed calcium phosphate scaffolds. *J Biomater Appl.* 2011 Sep;26:359–380.
55. Clarke MS, Sundaresan A, Vanderburg CR, et al. A three-dimensional tissue culture model of bone formation utilizing rotational co-culture of human adult osteoblasts and osteoclasts. *Acta Biomater.* 2013 Aug;9:7908–7916.
56. Han D, Zhang Q. An essential requirement for osteoclasts in refined bone-like tissue reconstruction in vitro. *Med Hypotheses.* 2006;67:75–78.
57. Hu K, Olsen BR. The roles of vascular endothelial growth factor in bone repair and regeneration. *Bone.* 2016 Oct;91:30–38.
58. Hu W, Chen Y, Dou C, et al. Microenvironment in subchondral bone: predominant regulator for the treatment of osteoarthritis. *Ann Rheum Dis.* 2020 Nov 6;80(4):413–422. <https://doi.org/10.1136/annrheumdis-2020-218089>. Epub ahead of print. PMID: 33158879; PMCID: PMC7958096.
59. Brinkmann J, Hefti T, Schlottig F, et al. Response of osteoclasts to titanium surfaces with increasing surface roughness: an in vitro study. *Biointerphases.* 2012 Dec;7:34.
60. Detsch R, Mayr H, Ziegler G. Formation of osteoclast-like cells on HA and TCP ceramics. *Acta Biomater.* 2008 Jan;4:139–148.
61. Shi R, Huang Y, Ma C, et al. Current advances for bone regeneration based on tissue engineering strategies. *Front Med.* 2019 Apr;13:160–188.