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Enhancement of osteoporotic fracture healing by vibration treatment: The role of osteocytes ☆

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ABSTRACT

The prevalence of osteoporotic fracture is high due to global aging problem. Delayed and impaired healing in osteoporotic fractures increase the socioeconomic burden significantly. Through intensive animal and clinical research in recent years, the pathogenesis of osteoporotic fracture healing is unveiled, including decreased inflammatory response, reduced mesenchymal stem cells and deteriorated angiogenesis, etc. The enhancement of osteoporotic fracture healing is important in shortening hospitalization, thus reducing related complications.

Mechanical stimulation is currently the most well-accepted approach for rehabilitation of osteoporotic fracture patients. Some new interventions providing mechanical signals were explored extensively in recent years, including vibration treatment, and osteoporotic fracture healing was found to respond very well to these signals. Vibration treatment could accelerate osteoporotic fracture healing with improved callus formation, mineralization and remodeling. However, the mechanism of how osteoporotic fracture bones sense mechanical signals and relay to bone formation remains unanswered.

Osteocytes are the most abundant cells in bone tissues. Cumulative evidence confirm that osteocyte is a type of mechanosensory cell and shows altered morphology and reduced cell density during aging. Meanwhile, osteocytes serve as endocrine cells to regulate bone and mineral homeostasis. However, the contribution of osteocytes in osteoporotic fracture healing is largely unknown. A recent *in vivo* study was conducted to examine the morphological and functional changes of osteocytes after vibration treatment in an osteoporotic metaphyseal fracture rat model. The findings demonstrated that vibration treatment induced significant outgrowth of canaliculi and altered expression of various proteins (E11, DMP1, FGF23 and sclerostin), particularly osteocyte-specific dentin matrix protein 1 (DMP1) which was greatly increased. DMP1 may play a major role in relaying mechanical signals to bone formation, which may require further experiments to consolidate. Most importantly, vibration treatment significantly increased the mineralization and accelerated the osteoporotic fracture healing in metaphyseal fracture model. In summary, osteocyte is the major cell type to sense mechanical signals and facilitate downstream healing in osteoporotic fracture bone. Vibration treatment has good potential to be translated for clinical application to benefit osteoporotic fracture patients, while randomized controlled trials are required to validate its efficacy.

Introduction

Aging population is rapidly increasing due to increase in life expectancy and decline in fertility rate. Osteoporotic fracture is a major global healthcare challenge due to escalating aging population, which is associated with substantial morbidity and mortality. There are 21 million men and 137 million women with a fracture probability above the threshold in the world for the year 2010, where 55% are from Asia [1]. According to International Osteoporosis

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Foundation (https://www.iofbonehealth.org/facts-statistics), 1 in 3 women and 1 in 5 men aged over 50 will experience osteoporotic fracture. 61% of osteoporotic fractures occur in women with a female-to-male ratio of 1.6 [2]. The most common fracture sites are distal radius, humerus, hip and spine fractures. Clinically, osteoporotic fractures are usually treated by surgical fixation, followed with weight-bearing walking for rehabilitation. To date, there is no promising medication to enhance osteoporotic fracture healing. Hence, mechanical loading is crucial for the rehabilitation of osteoporotic fracture healing; understanding the mechanism of how osteoporotic fracture bones sense mechanical signals and relay to bone formation is essential.

Pathology of impaired osteoporotic fracture healing

It has been reported that osteoporosis impairs fracture healing at different stages [3]. There are several known factors leading to deteriorated capacity of osteoporotic fracture healing, including decreased growth hormone [4], impaired inflammatory response [5], reduced mesenchymal stem cells [6], decreased angiogenesis [7,8], etc. Fracture healing is a complex physiological process involving spatial and temporal coordination of different cells, growth factors, proteins and genes. Therefore, any deterioration of these related factors induced by osteoporosis will ultimately cause poor mechanical environment of fracture sites and thus the fracture fixation [9,10]. These will lead to delayed and impaired fracture healing, with increased risk of complications. Enhancement of osteoporotic fracture healing is therefore in pressing need to reduce hospitalization period and occurrence of complications. Mechanical stimulation is the most common rehabilitation approach for fragility fracture patients, yet the detailed mechanism of how osteoporotic bones respond to mechanical signals to facilitate downstream fracture healing is largely unknown.

Roles of osteocytes in fracture healing

Osteocytes, residing in lacunae, comprise 90–95% of bone cells and are the longest lived bone cells. During aging, osteocytes die, leaving behind empty lacunae. Growing evidences reveal that osteocytes are mechanosensory cells as they can communicate with one another through their canaliculi [11]. Osteocytes also serve as endocrine cells to regulate bone and mineral homeostasis [12]. aging is associated with changes in osteocyte lacuna-canalicular network with respect to morphology, which lacunae becomes smaller and more spherical. The changes in osteocyte morphology and cell density will lead to changes in mechanosensitivity. Hence, there is load-adaptive response of osteocytes with aging [13]. Although there are many emerging research on the roles of osteocytes in aged bones, reports on their roles in fracture healing or particularly osteoporotic fracture healing are scarce. Recently, there is a systematic review reporting the latest update of this research area [14], revealing that there were only 23 related manuscripts up to October 2018.

The review summarized the spatial and temporal changes of osteocytes in the fracture healing process. During fracture which disrupts the cell-cell communication, osteocytes can trigger coordinated bone healing responses from programmed cell death of osteocytes at the acute phase and induce the recruitment of osteoclasts for bone resorption and remodeling. Haematoma is formed with the expression of pro-inflammatory markers cyclooxygenase-2 (Cox-2) and interleukin 6 (IL-6) at the early phase of fracture healing. This is followed by the expression of growth factors, bone morphogenetic protein (BMP)−2 and cysteine-rich angiogenic inducer 61 (CYR61) that match with neoangiogenesis, chondrogenesis and callus formation during the intermediate phase. The new vasculature helps to preserve the viability of osteocytes in the presence of vascular canals. Tightly controlled regulation of osteocyte-specific markers E11, dentin matrix protein 1 (DMP1) and sclerostin modulate and promote osteogenesis, mineralization and remodeling across different phases of fracture healing. These regulatory mechanisms signify the maturation of osteocytes and remodeling phase. During the healing process, BMP signaling for chondrogenesis and Wnt signaling for osteogenesis are regulated by osteocyte-secreted sclerostin. Interestingly, out of 23 papers included in the review, only two studied the roles of osteocytes in osteoporotic fracture healing with one related to sclerostin antibody treatment and one in fragility fracture patients. Whether osteocytes attribute to impaired healing capacity in osteoporotic bone is still unanswered [14] and need further research to address.

Mechanical stimulation enhances osteoporotic fracture healing

Weight-bearing walking is currently the most widely adapted rehabilitation approach to provide mechanical stimulation for fragility fracture patients. However, walking is difficult for fractured elderly, as this usually generates pain at fracture sites that will affect the compliance. In the meantime, there are many research attempting to search for novel interventions to enhance fracture healing that are usually easy to operate and pain-free. Low intensity pulsed ultrasound (LIPUS), extracorporeal shockwave (ESW), pulsed electromagnetic wave (PEMF), electrical stimulation (ES) are the modalities that were intensively studied before, where many of them (LIPUS, ESW) were mechanical related. However, not many studies investigated their efficacy on osteoporotic fracture healing. For LIPUS, Cheung et al. compared the LIPUS effect between ovariectomy(OVX)-induced osteoporotic and age-matched normal fracture healing. LIPUS was found to significantly increase callus width, bone volume fraction, endochondral ossification, and the stiffness in osteoporotic fracture that was comparable to the normal fracture group [15,16]. For ESW, Huang et al. compared the effect of focused ESW (0.26 mJ/mm^2, 60 doses/min, and 2000 pact quantities) on proximal tibial osteotomy between sham and OVX rats. Results indicated a promoted expression of osteoprotegerin (OPG) and BMP-2 in the osteoporotic fracture area by ESW [17]. Another similar study by Mackert et al. applied ESW on metaphyseal tibial fracture rat model. Results demonstrated that low-energy ESW (0.15 mJ/mm^2) with fewer treatment times could improve biomechanical properties, callus quantity and expression of bone transcription factors [18]. These evidences confirm the beneficial effect of mechanical stimulation on osteoporotic fracture healing, provided that the treatment settings are optimized. Meanwhile, these studies bear some limitations, such as using OVX to simulate osteoporosis status despite a FDA-approved model and some using diaphyseal fracture model that might not best reflect osteoporotic fracture clinically. Hence, the interpretation of these results need to be careful.

Effect of vibration treatment on osteoporotic fracture healing

Another mechanical modality used to promote fracture healing is vibration treatment. Vibration treatment provides physical oscillation at high frequency (20–90 Hz) and varied magnitude (low-magnitude <1 g or high-magnitude >1 g, where g = gravitational acceleration) to musculoskeletal system systemically. Vibration treatment has been reported to improve bone health, particularly in lumbar spine and femoral neck [19] and muscle performance [20]. Meanwhile, there are several research groups working on the efficacy of vibration treatment on fracture healing. Two recent systematic review articles confirmed the beneficial effect of vibration treatment on fracture healing without complications [21,22]. Wang reviewed 19 original articles, revealing that most of the studies showed positive effects of vibration treatment on fracture healing and the frequencies of 35 Hz and 50 Hz showed better results than others. In addition, ovariectomized animals gave

a better response to vibration (35–90 Hz; 0.3–10 g) than non-ovariectomized ones. Positive effects of vibration treatment on angiogenesis at fracture sites and surrounding muscles during fracture healing were reported in several studies [21]. Chen reviewed 9 eligible studies and found that vibration treatment could accelerate callus formation in early phase of bone healing, promote callus mineralization and maturation in later phase and restore mechanical properties of bones [22]. On the other hand, osteoporotic bone is generally believed to be less responsive to mechanical signals but these studies revealed that osteoporotic bones responded very well to mechanical loading. Two studies showed that estrogen receptor-α is crucial for the mechanostimulation of osteoporotic fracture healing by vibration treatment [23,24]. Therefore, vibration treatment has been well proven to be beneficial to osteoporotic and normal fracture healing, while the upstream sensing mechanism and translation to randomized controlled trial are the major directions to be investigated for future application.

Changes of osteocytes in osteoporotic fracture in response to vibration treatment and the role of dentin matrix protein 1 (DMP1)

As osteocyte is the major bone cell type in bone tissues and a mechanosensory cell, it may play a key role in sensing mechanical signals from vibration treatment and relaying to bone formation in order to facilitate fracture healing. An *in vivo* study was reported recently, in which morphological changes in the osteocyte lacuno-canalicular network (LCN) and mineralization of osteoporotic metaphyseal fracture model in response to vibration treatment were examined [25]. This is the first study to investigate the vibration effect on osteoporotic fracture healing in metaphyseal fracture model of Sprague-Dawley rats, as most of the previous similar studies used traditional diaphyseal fracture model [21,22]. Metaphyseal fracture model can simulate osteoporotic fracture much better and is more clinically relevant [26,27]. The study implemented a factorial design to compare 4 groups of Sham-CT, Sham-VT, OVX-CT, OVX-VT, where CT stands for control and VT stands for vibration. Radiography, histomorphometry, acid-etched scanning electron microscopy (SEM), energy-dispersive X-ray spectrometry analysis, confocal laser scanning microscopy, immunohistochemistry, microCT and mechanical testing were performed at day 7, 14 and 42 post-fracture [25].

The results showed that vibration treatment could enhance osteoporotic fracture healing in metaphyseal fracture model, as indicated by radiography, microarchitecture and mechanical properties. Interestingly, the enhancement in OVX group was comparable or even better than Sham group. This further confirms the positive effects of vibration treatment on osteoporotic fracture healing in both the diaphyseal or metaphyseal fracture models. In addition, LCN demonstrated a very remarkable outgrowth of dendrites (increase in dendritic branch points and canalicular length) in the OXV-VT group at day 14, as compared with OVX-CT group (Fig. 1). Vibration treatment could induce the expression of E11, dentin matrix protein 1 (DMP1) and fibroblast growth factor 23 (FGF23); in contrast, it downregulated the expression of sclerostin in osteocytes. Furthermore, vibration treatment enhanced mineralization, as reflected by significant increase in calcium-to-phosphorus (Ca-P) ratio, mineral apposition rate (MAR), mineralization surface (MS/BS) and bone mineral density (BMD) in OVX group [25]. This is the first and important evidence indicating that osteocyte is the major cell type sensing the mechanical signals from vibration treatment in osteoporotic fractured bone, resulting in the enhanced morphology and mineralization in order to accelerate the osteoporotic fracture healing, although the limitation of this study was that the osteocyte parameters were mainly quantified at the cortical bones while metaphyseal fracture healing should occur mostly in trabecular bones. In the study, osteocyte-specific DMP1 expression was also found to increase sharply by 66–97% in OVX group in response to vibration treatment at early phase (Day 7 and 14) [25], which may be a major protein relaying the mechanical signals to downstream bone formation. DMP1 knock-out or knock-down experiments are needed in the future to clarify its important role in osteoporotic fracture healing.

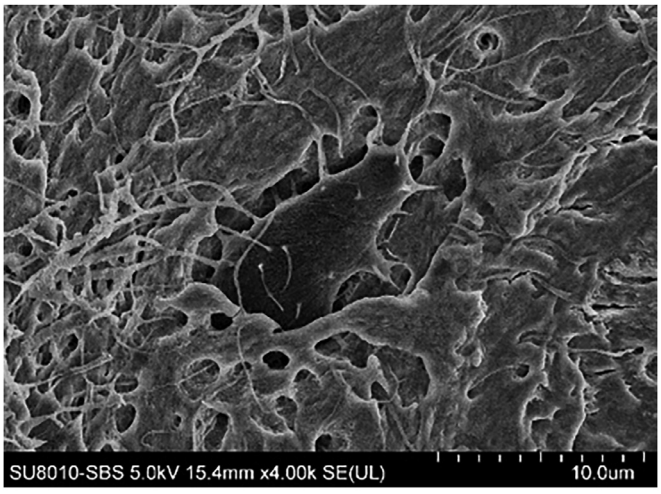

Fig. 1. Osteocyte lacuno-canalicular network (LCN) of **(A)** OVX-CT and **(B)** OVX-VT at Day 14 post-fracture. LCN demonstrated significant outgrowth of dendrites (increase in dendritic branch points and canalicular length) in the OXV-VT group than in the OVX-CT group.

Conclusion

Osteoporotic fracture is very common and a critical medical challenge that incurs substantial healthcare cost. Mechanical stimulation is currently the best rehabilitation approach for osteoporotic fracture patients. With searching for novel mechanical modalities to facilitate fracture healing, vibration treatment was shown to accelerate osteoporotic fracture healing through enhancing callus formation, bone remodeling and mineralization. Some mechanisms or pathways of how osteoporotic fractured bones respond to vibration treatment have been demonstrated, including enhancement of inflammatory response, angiogenesis, mesenchy-

mal stem cell recruitment and mechanical properties [5,7,28,29]. The upstream sensing mechanism was recently depicted, in which osteocyte lacuna-canalicular network plays a critical role. Dendrite outgrowth of osteocytes (increased dendritic branch points and canalicular length) were induced by vibration treatment, accompanied with the upregulation of various proteins (E11, DMP1, FGF23). Expression of DMP1, an osteocyte-specific protein, was particularly high; therefore it may play an important role in relaying mechanical signals to bone formation. The whole process of how osteoporotic fracture responds to vibration treatment has been well demonstrated. Vibration treatment has intriguing potential to be translated to clinical application. However, the animal data may not be directly applicable to clinical settings due to much more complicated conditions of clinical fracture cases, yet vibration treatment is a safe intervention as there is no complication reported clinically till now. Conducting randomized controlled trials is the next step to validate the efficacy of vibration treatment on fracture healing.

Declaration of Competing Interest

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

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References

[1] Oden A, McCloskey EV, Kanis JA, Harvey NC, Johansson H. Burden of high fracture probability worldwide: secular increases 2010-2040. Osteoporos Int 2015;26:2243–8.
[2] Johnell O, Kanis JA. An estimate of the worldwide prevalence and disability associated with osteoporotic fractures. Osteoporos Int 2006;17:1726–33.
[3] Pang J, Ye M, Gu X, Cao Y, Zheng Y, Guo H, et al. Ovariectomy-Induced Osteopenia Influences the Middle and Late Periods of Bone Healing in a Mouse Femoral Osteotomy Model. Rejuvenation Res 2015;18:356–65.
[4] Cheung WH, Miclau T, Chow SK, Yang FF, Alt V. Fracture healing in osteoporotic bone. Injury 2016;47(Suppl 2):S21–6.
[5] Chow SK, Chim YN, Wang J, Zhang N, Wong RM, Tang N, et al. Vibration treatment modulates macrophage polarisation and enhances early inflammatory response in oestrogen-deficient osteoporotic-fracture healing. Eur Cell Mater 2019;38:228–45.
[6] Josephson AM, Bradaschia-Correa V, Lee S, Leclerc K, Patel KS, Muinos Lopez E, et al. Age-related inflammation triggers skeletal stem/progenitor cell dysfunction. Proc Natl Acad Sci U S A 2019;116:6995–7004.
[7] Sun MH, Leung KS, Zheng YP, Huang YP, Wang LK, Qin L, et al. Three-dimensional high frequency power Doppler ultrasonography for the assessment of microvasculature during fracture healing in a rat model. J Orthop Res 2012;30:137–43.
[8] Cheung WH, Sun MH, Zheng YP, Chu WC, Leung AH, Qin L, et al. Stimulated angiogenesis for fracture healing augmented by low-magnitude, high-frequency vibration in a rat model-evaluation of pulsed-wave doppler, 3-D power Doppler ultrasonography and micro-CT microangiography. Ultrasound Med Biol 2012;38:2120–9.
[9] Augat P, Simon U, Liedert A, Claes L. Mechanics and mechano-biology of fracture healing in normal and osteoporotic bone. Osteoporos Int 2005;16(Suppl 2):S36–43.
[10] Hollensteiner M, Sandriesser S, Bliven E, von Rüden C, Augat P. Biomechanics of osteoporotic fracture fixation. Curr Osteoporos Rep 2019;17:363–74.
[11] Bonewald LF. The amazing osteocyte. J Bone Miner Res 2011;26:229–38.
[12] Dallas SL, Prideaux M, Bonewald LF. The osteocyte: an endocrine cell .. and more. Endocr Rev 2013;34:658–90.
[13] Hemmatian H, Bakker AD, Klein-Nulend J, van Lenthe GH. Aging, Osteocytes, and Mechanotransduction. Curr Osteoporos Rep 2017;15:401–11.
[14] Choy VMH, Wong RMY, Chow SKH, Li MC, Chim YN, Li TK, et al. How much do we know about the role of osteocytes in different phases of fracture healing? A systematic review. J Orthop Translat 2019.
[15] Cheung WH, Chow SK, Sun MH, Qin L, Leung KS. Low-intensity pulsed ultrasound accelerated callus formation, angiogenesis and callus remodeling in osteoporotic fracture healing. Ultrasound Med Biol 2011;37:231–8.
[16] Cheung WH, Chin WC, Qin L, Leung KS. Low intensity pulsed ultrasound enhances fracture healing in both ovariectomy-induced osteoporotic and age–matched normal bones. J Orthop Res 2012;30:129–36.
[17] Huang HM, Li XL, Tu SQ, Chen XF, Lu CC, Jiang LH. Effects of roughly focused extracorporeal shock waves therapy on the expressions of bone morphogenetic protein-2 and osteoprotegerin in osteoporotic fracture in rats. Chin Med J (Engl) 2016;129:2567–75.
[18] Mackert GA, Schulte M, Hirche C, Kotsougiani D, Vogelpohl J, Hoener B, et al. Low-energy extracorporeal shockwave therapy (ESWT) improves metaphyseal fracture healing in an osteoporotic rat model. PLoS ONE 2017;12:e0189356.
[19] Marin-Cascales E, Alcaraz PE, Ramos-Campo DJ, Martinez-Rodriguez A, Chung LH, Rubio-Arias JA. Whole-body vibration training and bone health in postmenopausal women: a systematic review and meta-analysis. Medicine (Baltimore) 2018;97:e11918.
[20] Lai CC, Tu YK, Wang TG, Huang YT, Chien KL. Effects of resistance training, endurance training and whole-body vibration on lean body mass, muscle strength and physical performance in older people: a systematic review and network meta-analysis. Age Ageing 2018;47:367–73.
[21] Wang J, Leung KS, Chow SK, Cheung WH. The effect of whole body vibration on fracture healing - a systematic review. Eur Cell Mater 2017;34:108–27.
[22] Chen J, Ruan H, Liu Y, Bao J, Xu H, Yao M, et al. Therapeutic effects of whole–body vibration on fracture healing in ovariectomized rats: a systematic review and meta-analysis. Menopause 2018;26:677–86.
[23] Haffner-Luntzer M, Kovtun A, Lackner I, Modinger Y, Hacker S, Liedert A, et al. Estrogen receptor alpha- (ERalpha), but not ERbeta-signaling, is crucially involved in mechanostimulation of bone fracture healing by whole-body vibration. Bone 2018;110:11–20.
[24] Chow SK, Leung KS, Qin J, Guo A, Sun M, Qin L, et al. Mechanical stimulation enhanced estrogen receptor expression and callus formation in diaphyseal long bone fracture healing in ovariectomy-induced osteoporotic rats. Osteoporos Int 2016;27:2989–3000.
[25] Choy MV, Wong RM, Li MC, Wang BY, Liu XD, Lee W, et al. Can we enhance osteoporotic metaphyseal fracture healing through enhancing ultrastructural and functional changes of osteocytes in cortical bone with low-magnitude high-frequency vibration? Faseb J. 2020.
[26] Wong RMY, Choy MHV, Li MCM, Leung KS, S KHC, Cheung WH, et al. A systematic review of current osteoporotic metaphyseal fracture animal models. Bone Joint Res 2018;7:6–11.
[27] Wong RM, Thormann U, Choy MH, Chim YN, Li MC, Wang JY, et al. A metaphyseal fracture rat model for mechanistic studies of osteoporotic bone healing. Eur Cell Mater 2019;37:420–30.
[28] Shi HF, Cheung WH, Qin L, Leung AH, Leung KS. Low-magnitude high-frequency vibration treatment augments fracture healing in ovariectomy-induced osteoporotic bone. Bone 2010;46:1299–305.
[29] Wei FY, Chow SK, Leung KS, Qin J, Guo A, Yu OL, et al. Low-magnitude high-frequency vibration enhanced mesenchymal stem cell recruitment in osteoporotic fracture healing through the SDF-1/CXCR4 pathway. Eur Cell Mater 2016;31:341–54.