Journal of Oral Biology and Craniofacial Research 9 (2019) 355–359

Contents lists available at ScienceDirect

Journal of Oral Biology and Craniofacial Research

journal homepage: www.elsevier.com/locate/jobcr

Mandible and iliac osteoblasts exhibit different Wnt signaling responses to LMHF vibration

Anute Pravitharangul[a], Srisurang Suttapreyasri[b], Chidchanok Leethanakul[c,*]

[a] Private Practice, Bangkok, 10700, Thailand
[b] Prince of Songkla University, Department of Oral Surgery, Faculty of Dentistry, Hat Yai, Songkhla, 90110, Thailand
[c] Prince of Songkla University, Orthodontic Section, Department of Preventive Dentistry, Faculty of Dentistry, Hat Yai, Songkhla, 90110, Thailand

ARTICLE INFO

Keywords:
Alkaline phosphatase
Catenin
Human
Mandible
Osteoblast
Vibration

ABSTRACT

Objective: The jaw bones and long bones have distinct developmental origins and respond differently to mechanical stimuli. This study aimed to compare the Wnt signaling responses of human mandible osteoblasts and long bone osteoblasts to low-magnitude, high-frequency (LMHF) mechanical vibration *in vitro*.

Methods: Primary human osteoblast cultures were prepared from mandibular bone ($n = 3$) and iliac bone ($n = 3$) specimens (six individuals). Osteoblast cell lines were subjected to vibration (0, 30, 60, 90, or 120 Hz) for 30 min. After 24 h, cells were vibrated for 30 min again, then harvested immediately to quantify *Wnt10b, Wnt5a* and runt-related transcription factor 2 (*RUNX2*) mRNA expression, β-catenin protein expression and alkaline phosphatase (ALP) activity.

Results: Mandible and iliac osteoblasts responded differently to LMHF vibration: *Wnt10b* mRNA was upregulated by the frequency range tested; *Wnt5a,* β-catenin protein expression and *RUNX2* mRNA expression were not altered. Furthermore, vibration upregulated ALP activity in mandible osteoblasts, but not in iliac osteoblasts.

Conclusions: This study demonstrates mandible osteoblasts and long bone osteoblasts respond differently to LMHF mechanical vibration in terms of Wnt signaling expression and ALP activity. Therefore, the effects of whole-body vibration on the long bones cannot be generalized to the jaw bones. Furthermore, osteoblast-like cells mediate the cellular responses to vibration, at least in part, by secreting extracellular signaling molecules.

1. Introduction

The components of the human skeleton derive from different embryological progenitor cells. Cranial bones, including the jaw bones, develop from neural crest cells, while other axial and appendicular bones are derived from mesodermal cells.[1] Even though bone is the common result, each embryonic cell lineage is associated with distinct bone formation and development processes, namely intramembranous and endochondral ossification. Bones from different developmental origins have distinct structures and cellular and extracellular matrix compositions, as well as varied patterns of growth factor expression and cellular responses to external stimuli.[2,3]

One of the primary functions of the skeleton is load bearing. Mechanical loading exerts a profound effect on bone cells. Interestingly, bone cells derived from different developmental origins exhibit varied responses to mechanical stimuli. Cyclic tensile strain increased glucose 6-phosphate dehydrogenase activity in rat long bone osteoblasts, but not in calvarial osteoblasts.[4] Compressive force upregulated the pro-inflammatory cytokines IL-1, IL-6, TNF-α and RANKL and down-regulated OPG in mandible osteoblasts.[5,6] In contrast, compression did not alter the expression of RANKL, OPG or the RANKL/OPG ratio in long bone osteoblasts.[7] These findings suggest the facial bones and long bones of the body respond differently to mechanical stimuli.

Low-magnitude, high-frequency (LMHF) mechanical vibration has been reported to exert anabolic effects on the long bones and has been clinically employed to prevent bone loss and increase bone mineral density in postmenopausal women.[8,9] In dentistry, LMHF mechanical vibration has been applied to accelerate tooth movement in patients undergoing orthodontic treatment.[10,11] Our recent study reported jaw bone osteoblasts and long bone osteoblasts responded differently to LMHF mechanical vibration; long bone osteoblasts exhibited a more osteogenic response than jaw bone osteoblasts.[12,13] In the present

Abbreviations: ALP, alkaline phosphatase; hIOBs, human iliac crest-derived mature osteoblast-like cells; hMOBs, human mandible-derived osteoblast-like cells; LMHF vibration, low-magnitude, high-frequency vibration; RUNX2, runt-related transcription factor 2

* Corresponding author.
E-mail addresses: chidchanok.l@psu.ac.th, nokleethanakul@yahoo.com (C. Leethanakul).

https://doi.org/10.1016/j.jobcr.2019.09.005
Received 26 May 2019; Received in revised form 9 August 2019; Accepted 30 September 2019
Available online 04 October 2019

study, we attempted to identify and clarify the signaling pathway underlying the disparate responses of long bone osteoblasts and jaw bone osteoblasts to LMHF vibration.

Wnt signaling plays significant role in osteoblast physiology and bone formation.[14] The canonical Wnt/β-catenin pathway is activated by mechanical stimuli such as compressive loading and vibration in osteoblastic cell lines.[15,16] Therefore, we hypothesized that jaw bone osteoblasts and long bone osteoblasts exposed to LMHF vibration would exhibit different Wnt signaling responses.

Thus, we compared the Wnt signaling responses of human mandible-derived osteoblast-like cells (hMOBs) and human iliac crest-derived osteoblast-like cells (hIOBs)—as models of jaw and long bone osteoblasts respectively—to various frequencies of LMHF mechanical vibration *in vitro*. The knowledge gained from this study may help to further characterize the pathways that regulate the distinct responses of jaw bones and long bones to vibrational stimuli.

2. Materials and methods

2.1. Preparation of human osteoblast-like cells

The Ethics Committee Board of Faculty of Dentistry, Prince of Songkla University, Thailand reviewed and approved the experimental procedures (EC6003-03-P-LR). Three mandibular bone specimens and three iliac crest bone specimens were derived from six individual patients, aged 18 to 25-years-old, undergoing orthognathic surgical procedures and iliac crest bone graft harvesting procedures. Primary osteoblast-like cell cultures were established from these bone specimens according to a previously published protocol.[12,17] Osteoblast-like cells in their third to fourth passages were used in this study. Each cell line was characterized to confirm their osteoblastic phenotype, as confirmed by strongly positive alizarin red and alkaline phosphatase staining over more than 70% of the plate.

Before the experiments, osteoblast-like cells were seeded at 2×10^5 cells/well in 6-well plates. After the cells reached 70% confluency, the medium was replaced with serum-free α-MEM for 12 h to synchronize the cell cycle, then changed to α-MEM containing 2% lactalbumin hydrolysate, 1% penicillin and 0.1% fungizone.

2.2. Application of LMHF mechanical vibration

Osteoblast-like cells were randomly allocated into one of five different frequency groups and mounted on a GJX-5 vibration calibrator (Beijing Sending Technology, Beijing, China) to receive vertical vibrational stimuli at 0, 30, 60, 90 or 120 Hz and a magnitude of 0.49 g for 30 min.

Cells were vibrated again after 24 h, then immediately harvested to quantify *Wnt10b, Wnt5a* and runt-related transcription factor 2 (*RUNX2*) mRNA expression, β-catenin protein expression and alkaline phosphatase (ALP) activity.

2.3. Real-time polymerase chain reaction

Total RNA was obtained and converted to cDNA according to a previously published protocol.[12] RT-PCR was performed by QuantStudio™ 5 Real-Time PCR System (Applied Biosystems™, Thermo Fisher Scientific, MA, USA) using the primer sequences listed in Table 1. Gene expression levels were calculated using the ΔΔCt method with Expression Suite Software (Version 1.1; Applied Biosystems™) using *GAPDH* as the reference gene.

2.4. Endogenous total β-catenin enzyme-linked immunosorbent assay

Total levels of endogenous β-catenin protein were quantified in osteoblast-like cell lysates using PathScan Total β-Catenin Sandwich ELISA kits (Cell Signaling Technology, Danvers, MA, USA). Total protein content was quantified using Pierce™ BCA Protein Assay Kit (Thermo Scientific, Massachusetts, U.S); Total β-catenin levels were normalized to the total protein content and presented as the relative amount.

Table 1
Primer sequences used for quantitative real-time polymerase chain reaction (qPCR).

Primer	Forward/ Reverse	Sequence (5′ - 3′)	Sequence ID
WNT5A	F	AATTCACAGAGGTGTTGCAGC	NM_003392.4
	R	TCAGGCACCATTAAACCACA	
WNT10B	F	AGACAGTGGTAGAGAGGTTT	NM_003394.3
	R	AGTATGATAAATTATCCCTT	
RUNX2	F	CAGATGGGACTGTGGTTACTGT	NM_001278478.1
	R	GTGAAGACGGTTATGGTCAAGG	
GAPDH	F	GCACCGTCAAGGCTGAGAAC	NM_001289746.1
	R	ATGGTGGTGAAGACGCCAGT	

2.5. Alkaline phosphatase activity assay

Alkaline phosphatase (ALP) activity was measured in osteoblast-like cell lysates using Alkaline Phosphatase Assay Kit (Abnova, Jhongli, Taiwan). ALP activity was normalized to total protein content and presented as the relative amount.

2.6. Statistical analysis

All data are presented as the mean and standard deviation of three independent cell lines assessed in triplicate. Kruskal-Wallis analysis and Bonferroni-type multiple comparison were used to assess differences between means with SPSS software, version 17.0 (SPSS, Chicago, IL, USA). Statistical significance was defined as $p < 0.05$.

3. Results

3.1. Vibration upregulates Wnt10B mRNA, but not Wnt5a mRNA, in hMOBs and hIOBs

Vibration significantly upregulated *Wnt10b* mRNA in hMOBs and hIOBs ($p < 0.05$, Fig. 1A). Human MOBs subjected to 60, 90 and 120 Hz vibration expressed significantly higher levels of *Wnt10b* than control cells, while lower frequency vibration (30, 60 and 90 Hz) significantly upregulated *Wnt10b* mRNA expression in hIOBs. In addition, hMOBs exposed to 30 and 60 Hz vibration expressed significantly lower levels of *Wnt10b* mRNA than hIOBs exposed to 30 and 60 Hz vibration, while hMOBs exposed to 120 Hz vibration expressed significantly higher levels of *Wnt10b* than hIOBs exposed to 120 Hz vibration.

Vibration did not alter *Wnt5a* mRNA expression in hMOBs or hIOBs, with the exception that hIOBs exposed to 120 Hz vibration expressed significantly more *Wnt5a* mRNA than hIOBs exposed to 0 Hz vibration and hMOBs exposed to 120 Hz vibration ($p < 0.05$, Fig. 1B).

3.2. Vibration does not affect total β-catenin protein expression or RUNX2 mRNA expression in hMOBs and hIOBs

Exposure to vibration at a range of frequencies did not significantly alter endogenous total β-catenin protein or *RUNX2* mRNA expression in hMOBs and hIOBs, with the exception that hIOBs exposed to 120 Hz vibration expressed a significantly higher level of *RUNX2* mRNA than hIOBs in the control 0 Hz group and hMOBs exposed to 120 Hz vibration ($p < 0.05$, Fig. 2A, B).

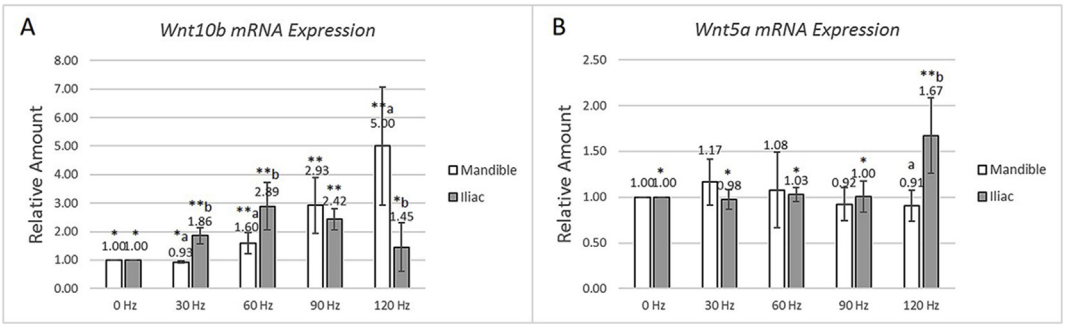

Fig. 1. Effect of LMHF vibration on the expression of canonical Wnt mRNAs in hMOBs and hIOBs. Cultured cells were exposed to 0, 30, 60, 90, or 120 Hz vibration for 30 min, cultured for 24 h, vibrated again for 30 min and then harvested. Real-time PCR analysis of A) *Wnt10b* and B) *Wnt5a* expression. Significant differences between different vibrational frequencies are indicated by *, ** and *** ($p < 0.05$, Kruskal-Wallis post-test). Significant differences between hMOBs and hIOBs are indicated by a and b ($p < 0.05$, Mann-Whitney U test).

3.3. Vibration increases ALP activity in hMOBs, but not in hIOBs

Vibration significantly increased ALP activity in hMOBs, but did not increase ALP activity in hIOBs ($p < 0.05$, Fig. 2C). Furthermore, human MOBs subjected to 90 Hz vibration had significantly higher ALP activity than hIOBs exposed to 90 Hz vibration.

3.4. Extracellular signaling molecules play a role in vibrational responses

To determine whether extracellular signaling molecules mediate the cellular effects of vibration, hMOBs and hIOBs were cultured for 24 h in the supernatant of hMOBs and hIOBs, respectively, that had been exposed to 0 Hz or 90 Hz vibration. Similarly to the direct effects of vibration on the osteoblast-like cell lines (Fig. 2C), culture in the supernatant of cells exposed to 90 Hz vibration significantly increased ALP activity in hMOBs compared to the supernatant of hMOBs exposed to 0 Hz vibration ($p < 0.05$, Fig. 3). In contrast, hIOBs cultured in the supernatant of hIOBs exposed to 0 or 90 Hz vibration had similar levels of ALP activity.

4. Discussion

This study aimed to compare the Wnt responses of human mandible osteoblasts and long bone osteoblasts to LMHF vibration. We quantified the mRNA levels of canonical and non-canonical Wnt signaling molecules; β-catenin, a key mediator of the canonical Wnt pathway; *RUNX2,* a related transcription factor, and the osteoblastic differentiation marker ALP in hMOBs and hIOBs exposed to various frequencies of vibration. Vibration differentially induced the expression of Wnt signaling molecules and upregulated ALP activity in hMOBs and hIOBs, but did not upregulate β-catenin protein expression or *RUNX2* mRNA in either type of osteoblast-like cell. Overall, this study demonstrates LMHF vibration induces different Wnt and ALP activity responses in mandible osteoblasts and iliac osteoblasts. Thus, straightforward generalization of the effects of whole-body vibration to the long bones to jaw bones should be avoided. Furthermore, osteoblast-like cells mediate the cellular responses to vibration, at least in part, by secreting extracellular signaling molecules.

Low-magnitude, high-frequency vibration with 30–125 Hz frequency range, magnitude 0.49 g and duration 30 min has been shown in

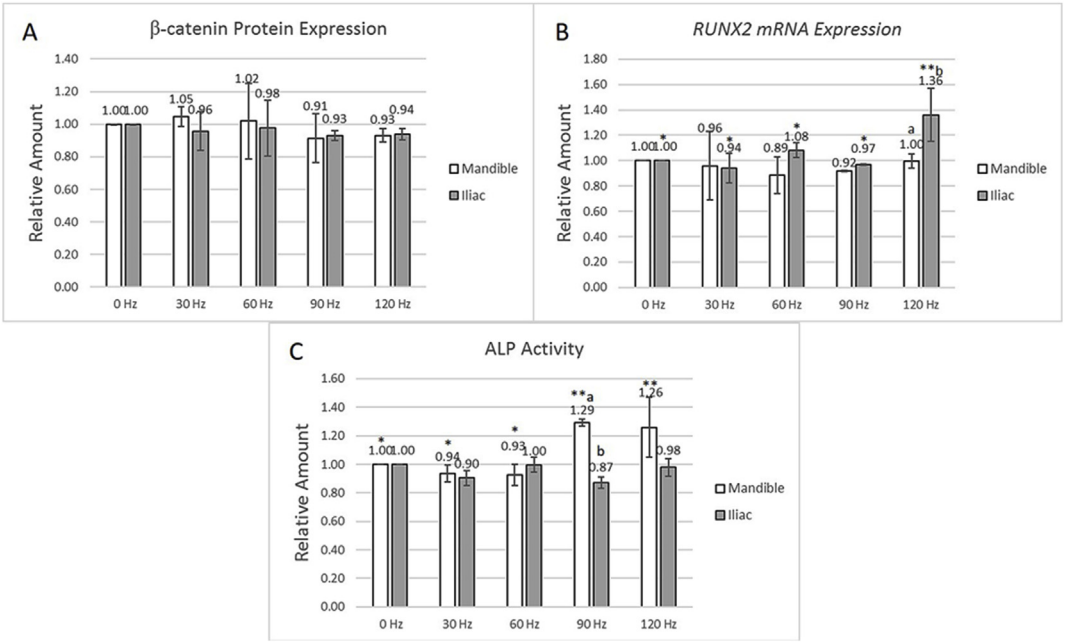

Fig. 2. Effect of LMHF mechanical vibration on A) β-catenin protein expression, B) runt-related transcription factor 2 *(RUNX2)* mRNA expression and C) alkaline phosphatase (ALP) activity in hMOBs and hIOBs. Cultured cells were exposed to 0, 30, 60, 90, or 120 Hz LMHF vibration for 30 min, cultured for 24 h, vibrated again for 30 min and then harvested. Significant differences between different vibrational frequency groups are indicated by * and ** ($p < 0.05$, Kruskal-Wallis post-test). Significant differences between hMOBs and hIOBs are indicated by a and b ($p < 0.05$, Mann-Whitney U test).

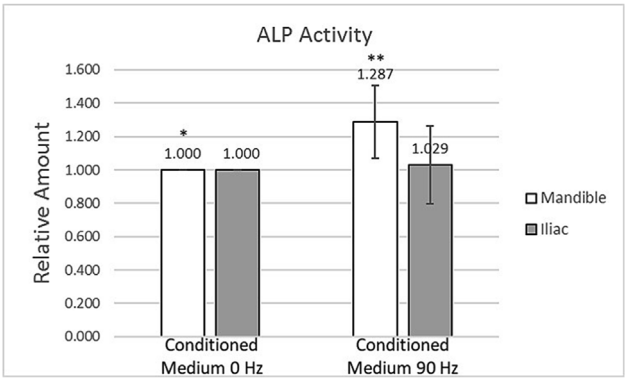

Fig. 3. Effect of conditioned medium from vibrated cells on alkaline phosphatase (ALP) activity in hMOBs and hIOBs. Human MOBs and hIOBs were cultured for 24 h in the supernatant of hMOBs and hIOBs, respectively, that had been exposed to vibration. The cells that were exposed to vibration were subjected to 0 or 90 Hz vibration for 30 min and the supernatant was collected 24 h later. Significant differences between different vibrational frequency groups are indicated by * and ** ($p < 0.05$, Kruskal-Wallis post-test).

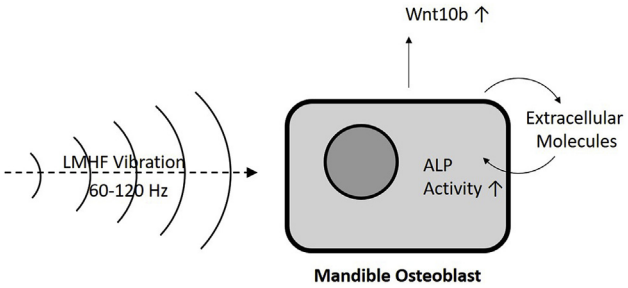

Fig. 4. The diagram represents the effects of low-magnitude, high-frequency mechanical vibration on human mandible osteoblasts.

previous clinical trials[10,11,18] and animal studies[19,20] that it significantly increased the rate of orthodontic tooth movement and rendered highest cellular responses in osteoblasts *in vitro*.[15,21] Thus, we used these vibrational parameters in our study.

Canonical Wnt signaling promotes osteoblastogenesis and exerts an inhibitory effect on osteoclast formation.[22] Canonical Wnt ligands, such as Wnt10B, induce accumulation of β-catenin in osteoblasts by inhibiting degradation of phosphorylated β-catenin through the ubiquitin-proteosome pathway. If allowed to accumulate, β-catenin is transported into the nucleus where it binds to TCF/LEF transcription factors and regulates *RUNX2* expression.[23] We found higher frequencies of LMHF vibration upregulated *Wnt10b* mRNA in both hMOBs and hIOBs. However, the effective frequency ranges were different for each cell type: 60–120 Hz for hMOBs and 30–90 Hz for hIOBs. These findings support the osteogenic effects of LMHF vibration and indicate the existence of specific effective frequency ranges for different types of bone. However, even though vibration upregulated Wnt10B ligand expression in hMOBs and hIOBs, the levels of β-catenin remained unaltered. This discrepancy could be related to the fact that rapid activation of Wnt signaling may only reduce the levels of phosphorylated β-catenin in the time frame examined in this study.

The vibration-induced alterations to canonical Wnt signaling observed in this study are consistent with a previous study that found exposure to 40 Hz vibrational stimuli for 14 days upregulated Wnt10b mRNA in rat long bone osteoblasts.[14] However, another recent study reported that rabbit calvarial osteoblasts subjected to 45 Hz vibrational stimuli for 3 days expressed higher levels of β-catenin protein.[24] These discrepancies might be related to the different time-points employed; we only assessed early responses (24 h) in this study, thus activation of β-catenin and RUNX2, which followed Wnt changes, might not yet occurred.

Non-canonical Wnt/Ca^{2+} signaling increases the intracellular Ca^{2+} level, activates nuclear factor of activated T cells (NFAT), and promotes osteoblast differentiation. Recently, non-canonical Wnt5a has been showed to be upregulated in inflammation[25] and stimulates production of pro-inflammatory cytokines such as IL-6 and IL-8 and enhance osteoclast formation in mouse cell cultures.[26] We found both hMOBs and hIOBs responded to vibration stimuli. Vibration upregulated canonical *Wnt10b* expression, but not non-canonical *Wnt5a* expression (though 120 Hz vibration did upregulate *Wnt5a* in hIOBs). These findings imply the upper limit of vibrational frequency for iliac osteoblasts should not be as high as 120 Hz.

Exposure to LMHF vibration did not affect the expression of *RUNX2* mRNA in hMOBs or hIOBs. However, LMHF vibration increased ALP activity in hMOBs, but not in hIOBs. These findings are consistent with a previous study that found culture in conditioned medium from vibrated osteocyte-like MLO-Y4 cells increased ALP activity in MC3T3-E1 mouse calvarial cells.[27] Additionally, human long bone osteoblasts subjected to 60 Hz vibration for 4 days had higher ALP activity.[21]

This study also indicates that the effects of vibration on osteoblast-like cells are mediated, at least in part, through induction of extracellular signaling molecules. Human MOBs cultured in the supernatant of cells vibrated at 90 Hz exhibited elevated ALP activity, similarly to the hMOBs that were directly exposed to 90 Hz vibration. This finding suggests exposure of osteoblasts to LMHF mechanical vibration induces the expression of extracellular mediators, which in turn mediate the effect of LMHF vibration in osteoblasts (Fig. 4).

This is the first study to compare the early Wnt signaling responses of human mandible and long bone osteoblasts to LMHF vibration. Primary osteoblast-like cells were utilized to make the model clinically relevant. Both canonical and non-canonical Wnt signaling molecules, along with β-catenin, the related transcription factor *Runx2* and the differentiation marker ALP were examined to make informative results. However, the osteoblast-like cell lines derived from each skeletal site were not obtained from the same subjects; therefore, interindividual variability may affect the results. Further studies are required to identify which extracellular signaling molecules are secreted by vibrated osteoblasts and provide more detailed insight knowledge of the mechanism underlying the responses to vibration.

In conclusion, hMOBs and hIOBs respond differently to LMHF mechanical vibration in terms of canonical Wnt ligand and ALP activity; hMOBs expressed higher levels of *Wnt10b* mRNAs and exhibited higher ALP activity than hIOBs. Therefore, the effects of whole-body vibration on the long bones cannot be generalized to the jaw bones. Furthermore, the vibrational responses of osteoblast-like cells may be mediated through extracellular signaling molecules.

Funding

The authors received no financial support.

Declaration of competing interest

The authors have no potential conflicts of interest with respect to the authorship and/or publication of this article to declare.

Acknowledgements

The authors gratefully acknowledge Prof. Dr. Prasit Pavasant for his helpful suggestions and thank the Cranio-Maxillofacial Hard Tissue Engineering Center of the Faculty of Dentistry, Prince of Songkla University for their research facilities.

References

1. Berendsen AD, Olsen BR. Bone development. *Bone.* 2015;80:14–18. https://doi.org/

10.1016/j.bone.2015.04.035 Epub 2015/10/11.
2. Akintoye SO, Lam T, Shi S, Brahim J, Collins MT, Robey PG. Skeletal site-specific characterization of orofacial and iliac crest human bone marrow stromal cells in same individuals. *Bone.* 2006;38(6):758–768. https://doi.org/10.1016/j.bone.2005.10.027.
3. Suttapreyasri S, Koontongkaew S, Phongdara A, Leggat U. Expression of bone morphogenetic proteins in normal human intramembranous and endochondral bones. *Int J Oral Maxillofac Surg.* 2006;35(5):444–452. https://doi.org/10.1016/j.ijom.2006.01.021.
4. Rawlinson SC, Mosley JR, Suswillo RF, Pitsillides AA, Lanyon LE. Calvarial and limb bone cells in organ and monolayer culture do not show the same early responses to dynamic mechanical strain. *J Bone Miner Res.* 1995;10(8):1225–1232. https://doi.org/10.1002/jbmr.5650100813.
5. Tripuwabhrut P, Mustafa M, Gjerde CG, Brudvik P, Mustafa K. Effect of compressive force on human osteoblast-like cells and bone remodelling: an in vitro study. *Arch Oral Biol.* 2013;58(7):826–836. https://doi.org/10.1016/j.archoralbio.2013.01.004.
6. Yamamoto K, Yamamoto T, Ichioka H, et al. Effects of mechanical stress on cytokine production in mandible-derived osteoblasts. *Oral Dis.* 2011;17(7):712–719. https://doi.org/10.1111/j.1601-0825.2011.01832.
7. Kreja L, Liedert A, Hasni S, Claes L, Ignatius A. Mechanical regulation of osteoclastic genes in human osteoblasts. *Biochem Biophys Res Commun.* 2008;368(3):582–587. https://doi.org/10.1016/j.bbrc.2008.01.106.
8. Slatkovska L, Alibhai SM, Beyene J, Hu H, Demaras A, Cheung AM. Effect of 12 months of whole-body vibration therapy on bone density and structure in postmenopausal women: a randomized trial. *Ann Intern Med.* 2011;155(10):668–679. https://doi.org/10.7326/0003-4819-155-10-201111150-00005.
9. Verschueren SM, Roelants M, Delecluse C, et al. Effect of 6-month whole body vibration training on hip density, muscle strength, and postural control in postmenopausal women: a randomized controlled pilot study. *J Bone Miner Res.* 2004;19(3):352–359.
10. Leethanakul C, Suamphan S, Jitpukdeebodintra S, Thongudomporn U, Charoemratrote C. Vibratory stimulation increases interleukin-1 beta secretion during orthodontic tooth movement. *Angle Orthod.* 2015;86(1):74–80. https://doi.org/10.2319/111914-830.1.
11. Cyclic loading (vibration) accelerates tooth movement in orthodontic patients: a double-blind, randomized controlled trial. In: Pavlin D, Anthony R, Raj V, Gakunga PT, eds. *Semin Orthod.* 2015. https://doi.org/10.1053/j.sodo.2015.06.005 Elsevier.
12. Pravitharangul A, Suttapreyasri S, Leethanakul C. Iliac and mandible osteoblasts exhibit varied responses to LMHF vibration. *Cell Biol Int.* 2018;42(10):1349–1357. https://doi.org/10.1002/cbin.11019.
13. Chatmahamongkol C, Pravitharangul A, Suttapreyasri S, Leethanakul C. The effect of compressive force combined with mechanical vibration on human alveolar bone osteoblasts. *J Oral Biol Craniofac Res.* 2019;9(1):81–85. https://doi.org/10.1016/j.jobcr.2018.10.003.
14. Chen B, Lin T, Yang X, et al. Low-magnitude, high-frequency vibration promotes the adhesion and the osteogenic differentiation of bone marrow-derived mesenchymal stem cells cultured on a hydroxyapatite-coated surface. *Int J Mol Med.* 2016;38(5):1531–1540.
15. Hou WW, Zhu ZL, Zhou Y, Zhang CX, Yu HY. Involvement of Wnt activation in the micromechanical vibration-enhanced osteogenic response of osteoblasts. *J Orthop Sci.* 2011;16(5):598. https://doi.org/10.1007/s00776-011-0124-5.
16. Sindhavajiva PR, Sastravaha P, Arksornnukit M, Pavasant P. Intermittent compressive force induces human mandibular-derived osteoblast differentiation via WNT/β-catenin signaling. *J Cell Biochem.* 2018;119(4):3474–3485. https://doi.org/10.1002/jcb.26519.
17. Gartland A, Buckley KA, Dillon JP, Curran JM, Hunt JA, Gallagher JA. *Isolation and Culture of Human Osteoblasts. Human Cell Culture Protocols.* Springer; 2005:29–54.
18. Kau CH, Nguyen JT, English. The clinical evaluation of a novel cyclical force generating device in orthodontics. *Orthod Pract.* 2010;1(1):10–15.
19. Nishimura M, Chiba M, Ohashi T, et al. Periodontal tissue activation by vibration: intermittent stimulation by resonance vibration accelerates experimental tooth movement in rats. *Am J Orthod Dentofacial Orthop.* 2008;133(4):572–583. https://doi.org/10.1016/j.ajodo.2006.01.046.
20. Alikhani M, Alansari S, Hamidaddin MA, et al. Vibration paradox in orthodontics: anabolic and catabolic effects. *PLoS One.* 2018;13(5):e0196540https://doi.org/10.1371/journal.pone.0196540.
21. Rosenberg N, Levy M, Francis M. Experimental model for stimulation of cultured human osteoblast-like cells by high frequency vibration. *Cytotechnology.* 2002;39(3):125–130https://dx.doi.org/10.1023%2FA%3A1023925230651.
22. Esen E, Chen J, Karner CM, Okunade AL, Patterson BW, Long F. WNT-LRP5 signaling induces Warburg effect through mTORC2 activation during osteoblast differentiation. *Cell Metabol.* 2013;17(5):745–755. https://doi.org/10.1016/j.cmet.2013.03.017.
23. Kobayashi Y, Maeda K, Takahashi N. Roles of Wnt signaling in bone formation and resorption. *Jpn Dent Sci Rev.* 2008;44(1):76–82. https://doi.org/10.1016/j.jdsr.2007.11.002.
24. Gao H, Zhai M, Wang P, et al. Low-level mechanical vibration enhances osteoblastogenesis via a canonical Wnt signaling-associated mechanism. *Mol Med Rep.* 2017;16(1):317–324. https://doi.org/10.3892/mmr.2017.6608.
25. Nakamura Y, Nawata M, Wakitani S. Expression profiles and functional analyses of Wnt-related genes in human joint disorders. *Am J Pathol.* 2005;167(1):97–105. https://doi.org/10.1016/S0002-9440(10)62957-4.
26. Maeda K. Wnt5a enhances RANKL-induced osteoclastogenesis. *J Bone Miner Res.* 2007;22(1):S43.
27. Wu XT, Sun LW, Qi HY, Shi H, Fan YB. The bio-response of osteocytes and its regulation on osteoblasts under vibration. *Cell Biol Int.* 2016;40(4):397–406. https://doi.org/10.1002/cbin.10575.