

The Aevo System™ utilizes Low-Intensity Pulsed Ultrasound (LIPUS) therapy to stimulate alveolar bone remodeling and accelerate orthodontic tooth movement.

The mechanism of action of LIPUS on alveolar bone remodelling <sup>(1-8)</sup> is to stimulate the osteoblast <sup>(9-14)</sup> and osteoclasts cells <sup>(15-22)</sup> independently. When LIPUS is applied to the bone cells, the mechanical stimuli are received by the cell receptors like integrins <sup>(23-28)</sup> and GPCR (G protein coupled receptors) <sup>(29-34)</sup> to activate different signaling pathways in the cells. The activation of these receptors begins the cell signaling cascade leading the nucleus of the cell to increase gene expression <sup>(35-42)</sup> which in turn leads to increased protein expression <sup>(43-47)</sup>. For osteoclasts, it increases RANKL (receptor activator of nuclear factor kappa B ligand) protein expression to accelerates bone resorption <sup>(48-53)</sup>. For osteoblasts, it increases bone forming proteins Runx2 (runt related transcription factor 2) <sup>(39, 54-56)</sup>, OPG (osteoprotegrin) <sup>(53, 57-63)</sup>, OCN (osteocalcin) <sup>(42, 64- 66)</sup>, and ALP (alkaline phosphatase) <sup>(65-73)</sup>.

#### References:

1. Yang B, Wu Q, Zhang L, Guo Y, Gong P. Effect of Low-intensity Pulsed Ultrasound on the Mandibular Remodeling Following Inferior Alveolar Nerve Transection. *Zhongguo Yi Xue Ke Xue Yuan Xue Bao*. 2017 Apr 20;39(2):215–24.
2. Alazzawi MMJ, Husein A, Alam MK, Hassan R, Shaari R, Azlina A, et al. Effect of low-level laser and low intensity pulsed ultrasound therapy on bone remodeling during orthodontic tooth movement in rats. *Prog Orthod*. 2018 Apr 16;19(1):10.
3. Lim K, Kim J, Seonwoo H, Park SH, Choung P-H, Chung JH. In vitro effects of low-intensity pulsed ultrasound stimulation on the osteogenic differentiation of human alveolar bone-derived mesenchymal stem cells for tooth tissue engineering. *BioMed Res Int*. 2013; 2013:269724.
4. Wang Y, Chai Z, Zhang Y, Deng F, Wang Z, Song J. Influence of low-intensity pulsed ultrasound on osteogenic tissue regeneration in a periodontal injury model: X-ray image alterations assessed by micro-computed tomography. *Ultrasonics*. 2014 Aug;54(6):1581–4.
- 5.\* Raza H, Dederich D, Major PW, El-Bialy T. Effect of Low Intensity Pulsed Ultrasound on Orthodontically Induced Root Resorption Caused by Torque: A Prospective Double Blinded Controlled Clinical Trial. 2015. 74 p.
6. Kang KL, Kim E-C, Park JB, Heo JS, Choi Y. High-Frequency, Low-Intensity Pulsed Ultrasound Enhances Alveolar Bone Healing of Extraction Sockets in Rats: A Pilot Study. *Ultrasound Med Biol*. 2016 Feb;42(2):493–502.
7. Kasahara Y, Usumi-Fujita R, Hosomichi J, Kaneko S, Ishida Y, Shibutani N, et al. Low-intensity pulsed ultrasound reduces periodontal atrophy in occlusal hypofunctional teeth. *Angle Orthod*. 2017 Sep;87(5):709–16.

8. El-Bialy T, El-Shamy I, Graber TM. Repair of orthodontically induced root resorption by ultrasound in humans. *Am J Orthod Dentofac Orthop Off Publ Am Assoc Orthod Its Const Soc Am Board Orthod*. 2004 Aug;126(2):186–93.
- 9.\* Alhazmi KS, El-Bialy T, Afify AR, Merdad LA, Hassan AH. Ultrasound Enhances Dentoalveolar Remodeling in an Ex Vivo Orthodontic, Ovariectomy-Induced Osteoporotic Model. *Ultrasound Med Biol*. 2017 Sep;43(9):1963–74.
10. Ishihara Y, Ueki K, Sotobori M, Marukawa K, Moroi A. Bone regeneration by statin and low-intensity pulsed ultrasound (LIPUS) in rabbit nasal bone. *J Cranio-Maxillo-fac Surg Off Publ Eur Assoc Cranio-Maxillo-fac Surg*. 2014 Apr;42(3):185–93.
11. Gu X-Q, Li Y-M, Guo J, Zhang L-H, Li D, Gai X-D. Effect of low intensity pulsed ultrasound on repairing the periodontal bone of Beagle canines. *Asian Pac J Trop Med*. 2014 Apr;7(4):325–8.
12. Hsu S-K, Huang W-T, Liu B-S, Li S-M, Chen H-T, Chang C-J. Effects of Near-Field Ultrasound Stimulation on New Bone Formation and Osseointegration of Dental Titanium Implants In Vitro and In Vivo. *Ultrasound Med Biol*. 2011 Mar;37(3):403–16.
13. Hsu S, Kuo C-C, Whu SW, Lin C-H, Tsai C-L. The effect of ultrasound stimulation versus bioreactors on neocartilage formation in tissue engineering scaffolds seeded with human chondrocytes in vitro. *Biomol Eng*. 2006 Oct;23(5):259–64.
14. Chen S-H, Chiu C-Y, Yeh J-M, Wang S-H. Effect of low intensity ultrasounds on the growth of osteoblasts. *Conf Proc Annu Int Conf IEEE Eng Med Biol Soc IEEE Eng Med Biol Soc Annu Conf*. 2007; 2007:5834–7.
15. Xie LK, Wangrangsimakul K, Suttapreyasri S, Cheung LK, Nuntanaranont T. A preliminary study of the effect of low intensity pulsed ultrasound on new bone formation during mandibular distraction osteogenesis in rabbits. *Int J Oral Maxillofac Surg*. 2011 Jul;40(7):730–6.
16. Chan CW, Qin L, Lee KM, Zhang M, Cheng JCY, Leung KS. Low intensity pulsed ultrasound accelerated bone remodeling during consolidation stage of distraction osteogenesis. *J Orthop Res Off Publ Orthop Res Soc*. 2006 Feb;24(2):263–70.
17. Ding Y, Li G, Zhang X, Ao J, Liu W, Ma Q, et al. Effect of low-intensity pulsed ultrasound on bone formation during mandible distraction osteogenesis in a canine model--a preliminary study. *J Oral Maxillofac Surg Off J Am Assoc Oral Maxillofac Surg*. 2009 Nov;67(11):2431–9.
- 18.\* Feres MFN, Kucharski C, Diar-Bakirly S, El-Bialy T. Effect of low-intensity pulsed ultrasound on the activity of osteoclasts: An in vitro study. *Arch Oral Biol*. 2016 Oct; 70:73–8.
19. Carina V, Costa V, Pagani S, De Luca A, Raimondi L, Bellavia D, et al. Inhibitory effects of low intensity pulsed ultrasound on osteoclastogenesis induced in vitro by breast cancer cells. *J Exp Clin Cancer Res CR*. 2018 Aug 20;37(1):197.
- 20.\* Al-Daghreer S, Doschak M, Sloan AJ, Major PW, Heo G, Scurtescu C, Tsui YY, El-Bialy t. Effect of low-intensity pulsed ultrasound on orthodontically induced root resorption in beagle dogs. *Ultrasound Med Biol*. 2014 Jun;40(6):1187–96.

21. Suzuki N, Hanmoto T, Yano S, Furusawa Y, Ikegame M, Tabuchi Y, et al. Low-intensity pulsed ultrasound induces apoptosis in osteoclasts: Fish scales are a suitable model for the analysis of bone metabolism by ultrasound. *Comp Biochem Physiol A Mol Integr Physiol*. 2016 May; 195:26–31.
22. Hanmoto T, Tabuchi Y, Ikegame M, Kondo T, Kitamura K-I, Endo M, et al. Effects of low-intensity pulsed ultrasound on osteoclasts: Analysis with goldfish scales as a model of bone. *Biomed Res Tokyo Jpn*. 2017;38(1):71–7.
23. Watabe H, Furuhashi T, Tani-Ishii N, Mikuni-Takagaki Y. Mechanotransduction activates  $\alpha_5\beta_1$  integrin and PI3K/Akt signaling pathways in mandibular osteoblasts. *Exp Cell Res*. 2011 Nov 1;317(18):2642–9.
24. Zhou S, Schmelz A, Seufferlein T, Li Y, Zhao J, Bachem MG. Molecular mechanisms of low intensity pulsed ultrasound in human skin fibroblasts. *J Biol Chem*. 2004 Dec 24;279(52):54463–9.
25. Cheng K, Xia P, Lin Q, Shen S, Gao M, Ren S, et al. Effects of low-intensity pulsed ultrasound on integrin-FAK-PI3K/Akt mechanochemical transduction in rabbit osteoarthritis chondrocytes. *Ultrasound Med Biol*. 2014 Jul;40(7):1609–18.
26. Xia P, Ren S, Lin Q, Cheng K, Shen S, Gao M, et al. Low-Intensity Pulsed Ultrasound Affects Chondrocyte Extracellular Matrix Production via an Integrin-Mediated p38 MAPK Signaling Pathway. *Ultrasound Med Biol*. 2015 Jun;41(6):1690–700.
27. Whitney NP, Lamb AC, Louw TM, Subramanian A. Integrin-mediated mechanotransduction pathway of low-intensity continuous ultrasound in human chondrocytes. *Ultrasound Med Biol*. 2012 Oct;38(10):1734–43.
28. Xia P, Wang X, Qu Y, Lin Q, Cheng K, Gao M, et al. TGF- $\beta$ 1-induced chondrogenesis of bone marrow mesenchymal stem cells is promoted by low-intensity pulsed ultrasound through the integrin-mTOR signaling pathway. *Stem Cell Res Ther*. 2017 Dec 13;8(1):281.
29. Matsumoto K, Shimo T, Kurio N, Okui T, Ibaragi S, Kunisada Y, et al. Low-intensity pulsed ultrasound stimulation promotes osteoblast differentiation through hedgehog signaling. *J Cell Biochem*. 2018 Jun;119(6):4352–60.
30. Padilla F, Puts R, Vico L, Raum K. Stimulation of bone repair with ultrasound: a review of the possible mechanic effects. *Ultrasonics*. 2014 Jul;54(5):1125–45.
31. Mahoney CM, Morgan MR, Harrison A, Humphries MJ, Bass MD. Therapeutic ultrasound bypasses canonical syndecan-4 signaling to activate rac1. *J Biol Chem*. 2009 Mar 27;284(13):8898–909.
32. Hou C-H, Tan T-W, Tang C-H. AMP-activated protein kinase is involved in COX-2 expression in response to ultrasound in cultured osteoblasts. *Cell Signal*. 2008 May;20(5):978–88.
- 33.\* Kaur H, Siraki AG, Uludağ H, Dederich DN, Flood P, El-Bialy T. Role of Reactive Oxygen Species during Low-Intensity Pulsed Ultrasound Application in MC-3 T3 E1 Pre-osteoblast Cell Culture. *Ultrasound Med Biol*. 2017 Nov;43(11):2699–712.

- 34.\* Kaur H, Siraki AG, Sharma M, Uludağ H, Dederich DN, Flood P, et al. Reactive Oxygen Species Mediate Therapeutic Ultrasound-Induced, Mitogen-Activated Protein Kinase Activation in C28/I2 Chondrocytes. *Ultrasound Med Biol*. 2018 Jul 21;
35. Li JK, Chang WH, Lin JC, Ruaan RC, Liu HC, Sun JS. Cytokine release from osteoblasts in response to ultrasound stimulation. *Biomaterials*. 2003 Jun;24(13):2379–85.
36. Ebisawa K, Hata K, Okada K, Kimata K, Ueda M, Torii S, et al. Ultrasound Enhances Transforming Growth Factor  $\beta$ -Mediated Chondrocyte Differentiation of Human Mesenchymal Stem Cells. *Tissue Eng*. 2004 May 1;10(5–6):921–9.
37. Fávaro-Pípi E, Bossini P, de Oliveira P, Ribeiro JU, Tim C, Parizotto NA, et al. Low-intensity pulsed ultrasound produced an increase of osteogenic genes expression during the process of bone healing in rats. *Ultrasound Med Biol*. 2010 Dec;36(12):2057–64.
38. Hou C-H, Hou S-M, Tang C-H. Ultrasound increased BMP-2 expression via PI3K, Akt, c-Fos/c-Jun, and AP-1 pathways in cultured osteoblasts. *J Cell Biochem*. 2009 Jan 1;106(1):7–15.
39. Akagi H, Nakanishi Y, Nakanishi K, Matsubara H, Hirose Y, Wang P-L, et al. Influence of Low-Intensity Pulsed Ultrasound Stimulation on Expression of Bone-Related Genes in Rat Bone Marrow Cells. *J Hard Tissue Biol*. 2016;25(1):1–5.
40. Sant'Anna EF, Leven RM, Viridi AS, Sumner DR. Effect of low intensity pulsed ultrasound and BMP-2 on rat bone marrow stromal cell gene expression. *J Orthop Res Off Publ Orthop Res Soc*. 2005 May;23(3):646–52.
41. Kusuyama J, Nakamura T, Ohnishi T, Eiraku N, Noguchi K, Matsuguchi T. Low-Intensity Pulsed Ultrasound (LIPUS) Promotes BMP9-Induced Osteogenesis and Suppresses Inflammatory Responses in Human Periodontal Ligament-Derived Stem Cells. *J Orthop Trauma*. 2017 Jul;31(7):S4.
42. Hasegawa T, Miwa M, Sakai Y, Niikura T, Kurosaka M, Komori T. Osteogenic activity of human fracture haematoma-derived progenitor cells is stimulated by low-intensity pulsed ultrasound in vitro. *J Bone Joint Surg Br*. 2009 Feb;91(2):264–70.
43. Tsai W-C, Pang J-HS, Hsu C-C, Chu N-K, Lin M-S, Hu C-F. Ultrasound stimulation of types I and III collagen expression of tendon cell and upregulation of transforming growth factor beta. *J Orthop Res Off Publ Orthop Res Soc*. 2006 Jun;24(6):1310–6.
44. Yang Z, Ren L, Deng F, Wang Z, Song J. Low-intensity pulsed ultrasound induces osteogenic differentiation of human periodontal ligament cells through activation of bone morphogenetic protein-smad signaling. *J Ultrasound Med Off J Am Inst Ultrasound Med*. 2014 May;33(5):865–73.
45. Wang F-S, Kuo Y-R, Wang C-J, Yang KD, Chang P-R, Huang Y-T, et al. Nitric oxide mediates ultrasound-induced hypoxia-inducible factor-1 $\alpha$  activation and vascular endothelial growth factor-A expression in human osteoblasts. *Bone*. 2004 Jul;35(1):114–23.
46. Scheven B a. A, Shelton RM, Cooper PR, Walmsley AD, Smith AJ. Therapeutic ultrasound for dental tissue repair. *Med Hypotheses*. 2009 Oct;73(4):591–3.

47. Scheven BA, Man J, Millard JL, Cooper PR, Lea SC, Walmsley AD, et al. VEGF and odontoblast-like cells: stimulation by low frequency ultrasound. *Arch Oral Biol.* 2009 Feb;54(2):185–91.
48. Chiu C-Y, Tsai T-L, Vanderby R, Bradica G, Lou S-L, Li W-J. Osteoblastogenesis of Mesenchymal Stem Cells in 3-D Culture Enhanced by Low-Intensity Pulsed Ultrasound through Soluble Receptor Activator of Nuclear Factor Kappa B Ligand. *Ultrasound Med Biol.* 2015 Jul;41(7):1842–52.
49. Rego EB, Inubushi T, Miyauchi M, Kawazoe A, Tanaka E, Takata T, et al. Ultrasound stimulation attenuates root resorption of rat replanted molars and impairs tumor necrosis factor- $\alpha$  signaling in vitro. *J Periodontal Res.* 2011 Dec;46(6):648–54.
50. Bandow K, Nishikawa Y, Ohnishi T, Kakimoto K, Soejima K, Iwabuchi S, et al. Low-intensity pulsed ultrasound (LIPUS) induces RANKL, MCP-1, and MIP-1 $\beta$  expression in osteoblasts through the angiotensin II type 1 receptor. *J Cell Physiol.* 2007 May;211(2):392–8.
51. Liu Z, Xu J, E L, Wang D. Ultrasound enhances the healing of orthodontically induced root resorption in rats. *Angle Orthod.* 2012 Jan;82(1):48–55.
- 52.\* El-Bialy T, Lam B, Aldaghreer S, Sloan AJ. The effect of low intensity pulsed ultrasound in a 3D ex vivo orthodontic model. *J Dent.* 2011 Oct;39(10):693–9.
53. Inubushi T, Tanaka E, Rego EB, Ohtani J, Kawazoe A, Tanne K, et al. Ultrasound stimulation attenuates resorption of tooth root induced by experimental force application. *Bone.* 2013 Apr;53(2):497–506.
54. Takayama T, Suzuki N, Ikeda K, Shimada T, Suzuki A, Maeno M, et al. Low-intensity pulsed ultrasound stimulates osteogenic differentiation in ROS 17/2.8 cells. *Life Sci.* 2007 Feb 13;80(10):965–71.
55. Chen Y-J, Wang C-J, Yang KD, Chang P-R, Huang H-C, Huang Y-T, et al. Pertussis toxin-sensitive G $\alpha$  protein and ERK-dependent pathways mediate ultrasound promotion of osteogenic transcription in human osteoblasts. *FEBS Lett.* 2003 Nov 6;554(1–2):154–8.
56. Suzuki A, Takayama T, Suzuki N, Sato M, Fukuda T, Ito K. Daily low-intensity pulsed ultrasound-mediated osteogenic differentiation in rat osteoblasts. *Acta Biochim Biophys Sin.* 2009 Feb;41(2):108–15.
57. Dalla-Bona DA, Tanaka E, Inubushi T, Oka H, Ohta A, Okada H, et al. Cementoblast response to low- and high-intensity ultrasound. *Arch Oral Biol.* 2008 Apr;53(4):318–23.
58. Borsje MA, Ren Y, de Haan-Visser HW, Kuijjer R. Comparison of low-intensity pulsed ultrasound and pulsed electromagnetic field treatments on OPG and RANKL expression in human osteoblast-like cells. *Angle Orthod.* 2010 May;80(3):498–503.
59. Liu Z, Xu J, E L, Wang D. Ultrasound enhances the healing of orthodontically induced root resorption in rats. *Angle Orthod.* 2012 Jan;82(1):48–55.
60. Renno ACM, Fávoro-Pípi E, Fernandes K, Tim C, Ribeiro DA. Ultrasound therapy modulates osteocalcin expression during bone repair in rats. *Ultrasonics.* 2012 Jan;52(1):111–6.

61. Tanaka E, Kuroda S, Horiuchi S, Tabata A, El-Bialy T. Low-intensity pulsed ultrasound in dentofacial tissue engineering. *Ann Biomed Eng.* 2015 Apr;43(4):871–86.
- 62.\* Al-Daghreer, Doschak M, Sloan AJ, Major PW, Heo G, Scurtescu C, Tsui YY, El-Bialy T. Long term effect of low intensity pulsed ultrasound on human tooth slice organ culture. *Archives of Oral Biology.* 2012; 57(6): 760 - 768.
- 63.\* Al-Daghreer, Doschak M, Sloan AJ, Major PW, Heo G, Scurtescu C, Tsui YY, El-Bialy T. Short term effect of low intensity pulsed ultrasound on an ex-vivo tooth culture. *Ultrasound Med Biol.* 2013; 39 (6): 1066 - 1074.
64. Warden SJ, Favaloro JM, Bennell KL, McMeeken JM, Ng KW, Zajac JD, et al. Low-intensity pulsed ultrasound stimulates a bone-forming response in UMR-106 cells. *Biochem Biophys Res Commun.* 2001 Aug 24;286(3):443–50.
65. Leung KS, Cheung WH, Zhang C, Lee KM, Lo HK. Low intensity pulsed ultrasound stimulates osteogenic activity of human periosteal cells. *Clin Orthop.* 2004 Jan;(418):253–9.
66. Harle J, Salih V, Knowles JC, Mayia F, Olsen I. Effects of therapeutic ultrasound on osteoblast gene expression. *J Mater Sci Mater Med.* 2001 Dec;12(10–12):1001–4.
- 67.\* Mostafa NZ, Uludağ H, Dederich DN, Doschak MR, El-Bialy TH. Anabolic effects of low-intensity pulsed ultrasound on human gingival fibroblasts. *Arch Oral Biol.* 2009 Aug;54(8):743–8.
68. Yang R-S, Lin W-L, Chen Y-Z, Tang C-H, Huang T-H, Lu B-Y, et al. Regulation by ultrasound treatment on the integrin expression and differentiation of osteoblasts. *Bone.* 2005 Feb;36(2):276–83.
69. Angle SR, Sena K, Sumner DR, Viridi AS. Osteogenic differentiation of rat bone marrow stromal cells by various intensities of low-intensity pulsed ultrasound. *Ultrasonics.* 2011 Apr;51(3):281–8.
70. Uddin SMZ, Qin Y-X. Enhancement of osteogenic differentiation and proliferation in human mesenchymal stem cells by a modified low intensity ultrasound stimulation under simulated microgravity. *PloS One.* 2013;8(9):e73914.
71. Pomini KT, Andreo JC, Rodrigues A de C, de O Gonçalves JB, Daré LR, German IJS, et al. Effect of low-intensity pulsed ultrasound on bone regeneration: biochemical and radiologic analyses. *J Ultrasound Med Off J Am Inst Ultrasound Med.* 2014 Apr;33(4):713–7.
72. Sun JS, Hong RC, Chang WH, Chen LT, Lin FH, Liu HC. In vitro effects of low-intensity ultrasound stimulation on the bone cells. *J Biomed Mater Res.* 2001 Dec 5;57(3):449–56.
73. Unsworth J, Kaneez S, Harris S, Ridgway J, Fenwick S, Chenery D, et al. Pulsed low intensity ultrasound enhances mineralisation in preosteoblast cells. *Ultrasound Med Biol.* 2007 Sep;33(9):1468–74.

\*: Study used the actual or a prototype of the Aevo System