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# The effects of gentle micropulse vibration on two types of murine osteoblasts

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Casa abierta al tiempo
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# Introduction.

- One today’s greatest challenges in orthodontics is reducing treatment time. Different attempts have been made to accelerate orthodontic tooth movement. The development of low-magnitude and high-frequency vibrations have gained interest within mechanical loading, because evidence has shown that it regulates bone homeostasis. Acceledent Aura™ has been developed to use cyclic forces and vibrations, which have been shown to accelerate tooth movement from 15% to 30% in animal experiments (1), and in patients have achieved 2 to 3 mm/month of tooth movement using the micro pulse vibration rate from 20 Hz to 30 Hz for 20 min/day (2).
- Within the biology of orthodontic tooth movement, it has been shown that there is a synthesis of cytokines expression (3) which has been stimulated by mechanical loading (4, 5). The expression of IL-1 β, TNF-α and IL-6 mRNAs has been shown to be up-regulated in both PDL cells and osteoblasts on the compression side during orthodontic tooth movement (6, 7) as collagenase-2 (MMP-8), collagenase-3 (MMP-13), TIMP-1, and TIMP-2 expression, (8, 9).

# Introduction.

- Both RANKL and OPG mRNAs are widely expressed in osteoblasts and PDL cells throughout the periodontal tissues (10, 11) that constituents of a ligand–receptor system known as the RANK/RANKL/OPG triad that directly regulates the final steps of the bone resorptive cascade, that allows tooth movement. IL-4 regulates bone homeostasis by direct effect on osteoclasts by inhibiting mature osteoclasts function through interfering with NF-κB and $Ca^{2+}$ signaling (12) suppressing RANK mRNA expression in osteoclastic precursors cells (13). IL-4 and IL-13 have been also shown to increase OPG expression in osteoblasts (14). IL-17 induces differentiation and function of osteoclasts which upregulate cathepsin K and MMP-9 expression an effect mediated by PGE(2) (15). Although RANKL induce osteoclast differentiation, TGF-β is a co-stimulator of the differentiation and survival of the osteoclastic cells, a soluble factor expressed by osteoblasts (16). In the present study, we hypothesized that osteoblasts are able to sense low-magnitude, high-frequency vibrations that may influence bone remodeling regulation.

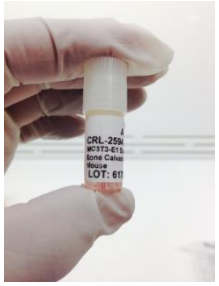
# Aim

- The purpose of this study was to further understand the role of the osteoblastic cell signaling molecules IL-4, IL-13, IL-17, OPG, sRANKL and TGF-β stimulated with micropulse vibration using the Acceledent Aura device.

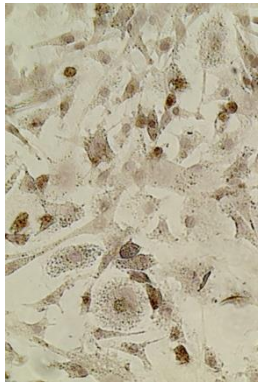
# Material and Method.

- Primary mouse osteoblasts M3T3 Subclone 14 (ATCC) and primary Calvarial mouse Balb/c osteoblasts were cultivated *in vitro* and subjected to gentle micropulse vibration (0.25 N; 30Hz) with the AcceleDent® aura appliance (Ortho Accel Technologies Inc. Bellaire, Texas, USA)(N=5), assayed before to start the experiments and after 20 minutes of stimulation with the appliance for IL-14, IL-13, IL-17, OPG, soluble RANKL and TGF-β protein by enzyme-linked immunosorbent assays (ELISAs; R&D Systems, Minneapolis, MN, USA; Peprotech, USA ) (Figure 1).

# Material and Method.

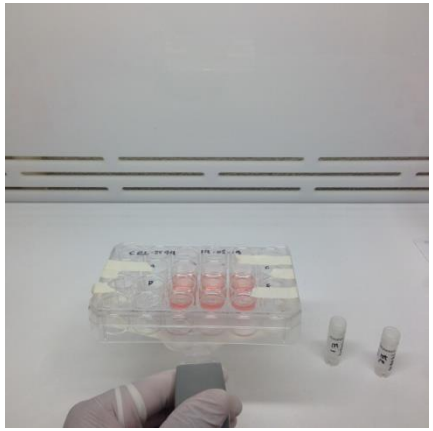


ATCC mouse osteoblasts



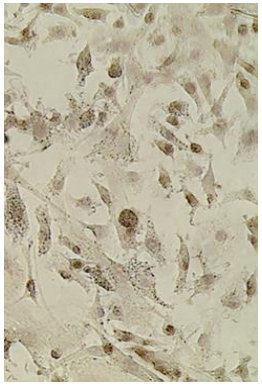
Primary mouse osteoblasts M3T3 Subclone 14

Micro pulse vibration on both types of osteoblasts



Enzyme-linked immunosorbent assays

Balb/C new born mice



Primary Calvarial mouse osteoblasts

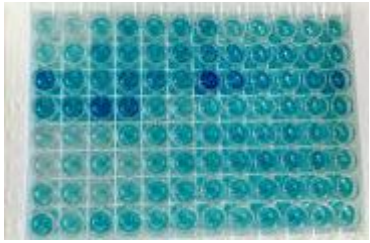


Figure 1

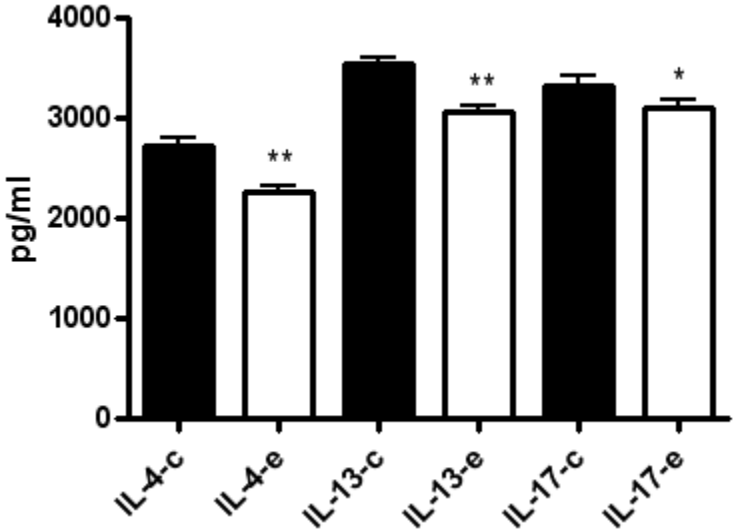
# Statistics.

- Data is expressed as mean standard error of the mean (SEM). Differences between control and experimental cultures were determined by the U-Mann Whitney U test, using Graph Pad Prism 4 software (Graph Pad Software Inc., San Diego, CA,USA) and the level of significance set at $P<0.05$.

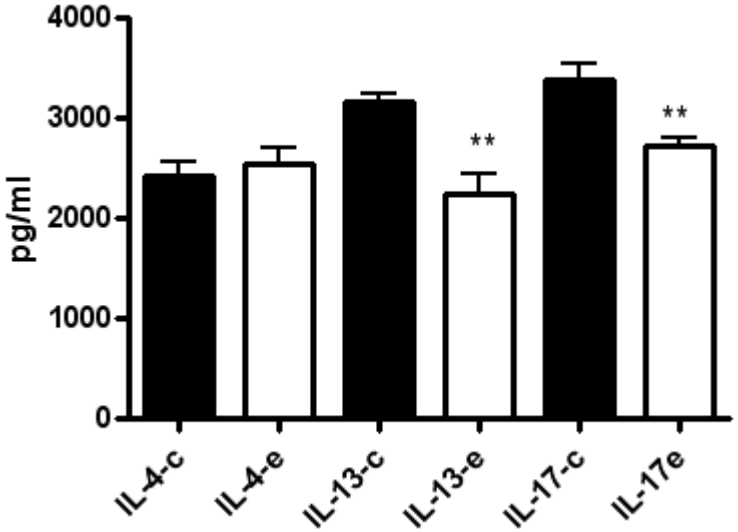
# Results.

## Effects of micropulse vibration on cytokine synthesis.

Graph 1.



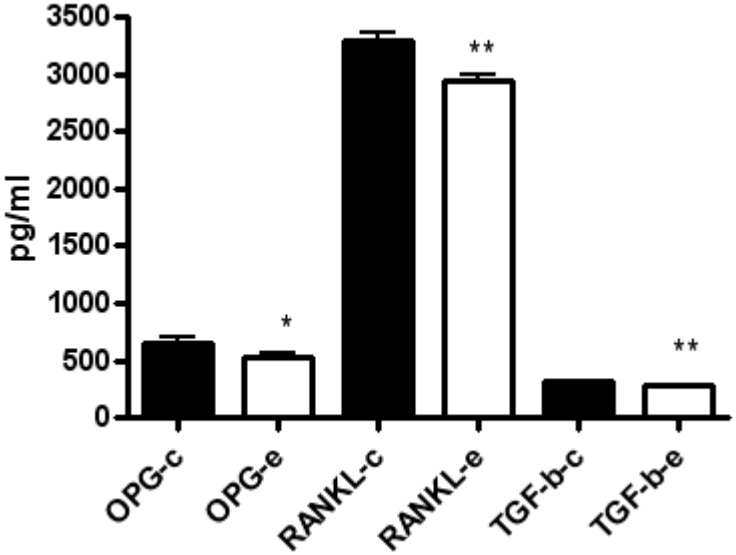

Graph 2.



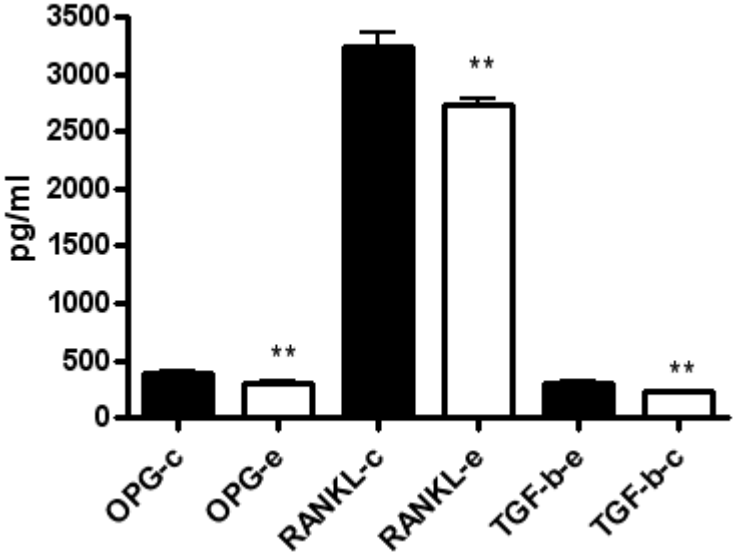

**Graph 1.** Cytokine production by mouse osteoblast MC3T3-E1 Subclone 14 (ATCC$^{R}$-CRL-2594$^{TM}$). **Graph 2.** Calvarial osteoblasts. Both types of osteoblasts in monolayer culture were subjected to micro pulse vibration of 0.25 N; 30Hz and the culture media assayed before and after the time of course experiments for OPG, sRANKL and TGF-β by ELISAs. Results are expressed as mean ±SEM for 5 cultures. Experimental significantly down-regulated than control. $P < 0.05$ is *; $P < 0.01$ is ** and $P < 0.001$ ***

# Results.

Graph 3.




Graph 4.




**Graph 3.** Cytokine production by mouse osteoblast MC3T3-E1 Subclone 14 (ATCC$^{R}$-CRL-2594$^{TM}$). **Graph 4.** Calvarial osteoblasts. Both types of osteoblasts in monolayer culture were subjected to micro pulse vibration of 0.25 N; 30Hz and the culture media assayed before and after the time of course experiments for OPG, sRANKL and TGF-β by ELISAs. Results are expressed as mean ±SEM for 5 cultures. Experimental significantly down-regulated than control. $P < 0.05$ is *; $P < 0.01$ is ** and $P < 0.001$ ***

# Discussion

- Since the work developed by Wölff (1892), it has been considered that physical loading induces bone remodeling. Recently it has been hypothesized that small physical stimuli, at sufficiently high, but physiologically relevant, frequencies can be critical determinants of bone morphology (17) and thus represent a unique means of mediating bone quantity and quality. High-frequency, low-magnitude signals have been shown to successfully stimulate an increase in cortical bone (18, 19). This data supports the premise that extremely small mechanical signals may also be capable of serving as a regulatory influence on bone cells, which seems to be distributed in bone under load (20), that represent both an endogenous anabolic stimulus to bone tissue (21) and an antiresorptive factor that can actively inhibit osteoclastogenesis (22).

# Conclusion.

This study has shown that mouse osteoblast MC3T3-E1 Subclone 14 (ATCC$^{R}$-CRL-2594™ ) and murin calvarial osteoblasts are able to sense to gentle micropulse vibration using the Acceledent Aura device, may be involved by producing soluble factors that contribute to bone remodeling regulation.

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