ORIGINAL ARTICLE

Periodontal tissue activation by vibration: Intermittent stimulation by resonance vibration accelerates experimental tooth movement in rats

Makoto Nishimura,[a] Mirei Chiba,[b] Toshiro Ohashi,[c] Masaaki Sato,[d] Yoshiyuki Shimizu,[e] Kaoru Igarashi,[f] and Hideo Mitani[g]

Sendai and Fukushima, Japan

Introduction: Accelerating the speed of orthodontic tooth movement should contribute to the shortening of the treatment period. This would be beneficial because long treatment times are a negative aspect of orthodontic treatment. In this study, we evaluated the effects of mechanical stimulation by resonance vibration on tooth movement, and we showed the cellular and molecular mechanisms of periodontal ligament responses. **Methods:** The maxillary first molars of 6-week-old male Wistar rats were moved to the buccal side by using an expansive spring for 21 days (n = 6, control group), and the amount of tooth movement was measured. Additional vibrational stimulation (60 Hz, 1.0 m/s^2) was applied to the first molars by using a loading vibration system for 8 minutes on days 0, 7, and 14 during orthodontic tooth movement (n = 6, experimental group). The animals were killed under anesthesia, and each maxilla was dissected. The specimens were fixed, decalcified, and embedded in paraffin. Sections were used for immunohistochemical analysis of receptor activator of NF kappa B ligand (RANKL) expression. The number of osteoclasts in the alveolar bone was counted by using TRAP staining, and the amount of root resorption was measured in sections stained with hematoxylin and eosin. **Results:** The average resonance frequency of the maxillary first molar was 61.02 ± 8.38 Hz. Tooth movement in the experimental group was significantly greater than in the control group ($P < .05$). Enhanced RANKL expression was observed at fibroblasts and osteoclasts in the periodontal ligament of the experimental group on day 3. The number of osteoclasts in the experimental group was significantly increased over the control group on day 8 ($P < .05$). Histologically, there were no pathological findings in either group or significant differences in the amount of root resorption between the 2 groups. **Conclusions:** The application of resonance vibration might accelerate orthodontic tooth movement via enhanced RANKL expression in the periodontal ligament without additional damage to periodontal tissues such as root resorption. (Am J Orthod Dentofacial Orthop 2008;133:572-83)

Orthodontic tooth movement is generated by the coupling of bone resorption on the compressed side of the periodontal ligament (PDL) and by bone formation on the stretched side of the PDL as a consequence of therapeutic mechanical stress. Since orthodontic treatment usually takes place over a long period of time, the problems of caries, periodontal disease, and prolonged treatment period are burdensome for the patient. Furthermore, it has been reported that total treatment duration proves to be highly correlated with root resorption.[1] In this respect, it is important to accelerate alveolar bone remodeling during orthodontic treatment, to shorten the time required for successful therapy.

To date, to accelerate tooth movement, physical

[a]Postdoctor fellow, Division of Oral Dysfunction Science, Department of Oral Health and Development Sciences, Graduate School of Dentistry, Tohoku University, Sendai, Japan.
[b]Associate professor, Division of Oral Dysfunction Science, Department of Oral Health and Development Sciences, Graduate School of Dentistry, Tohoku University, Sendai, Japan.
[c]Associate professor, Biomechanics Laboratory, Department of Bioengineering and Robotics, Graduate School of Engineering, Tohoku University, Sendai, Japan.
[d]Professor, Biomechanics Laboratory, Department of Bioengineering and Robotics, Graduate School of Engineering, Tohoku University, Sendai, Japan.
[e]Private practice, Fukushima, Japan.
[f]Professor, Division of Oral Dysfunction Science, Department of Oral Health and Development Sciences, Graduate School of Dentistry, Tohoku University, Sendai, Japan.
[g]Professor emeritus, Tohoku University, Sendai, Japan.

Supported in part by a grant-in-aid for scientific research (B) from the Ministry of Education, Science, Sports, and Culture of Japan (Nos. 12557179, 16390602).

Reprint requests to: Mirei Chiba, Division of Oral Dysfunction Science, Department of Oral Health and Development Sciences, Graduate School of Dentistry, Tohoku University, 4-1 Seiryo-machi, Aoba-ku, Sendai 980-8575, Japan; e-mail, mirei@mail.tains.tohoku.ac.jp.

Submitted, July 2005; revised and accepted, January 2006.
0889-5406/$34.00

doi:10.1016/j.ajodo.2006.01.046

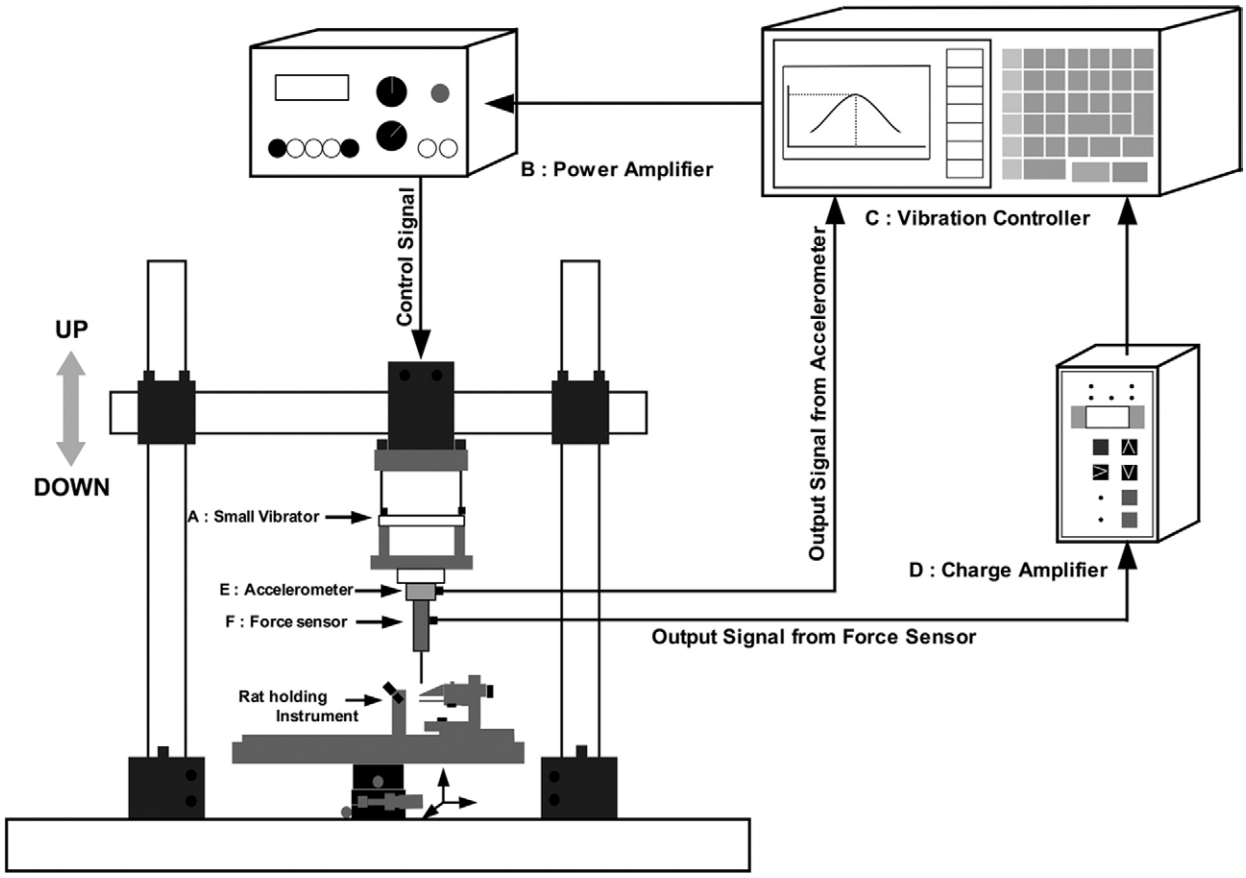

Fig 1. Resonance vibration delivery system: *A,* small vibrator; *B,* power amplifier; *C,* vibration controller; *D,* charge amplifier; *E,* accelerometer; *F,* force sensor.

approaches with low-energy laser irradiation[2] and magnetic fields,[3,4] as well as pharmacological approaches with the injection of prostaglandin E_2 (PGE_2)[5-7] and 1,25-$(OH)_2D_3$[8-10] during tooth movement, have been investigated. However, many side effects, such as local pain, severe root resorption,[11] and drug-induced side effects[12] have been reported.

The initial response of cells to mechanical stress in vitro appears within 30 minutes.[13-15] We have attempted to activate these initial responses at the cellular level by applying resonance vibrational stimulation to a tooth and its periodontal tissue. Moreover, the loading of resonance vibration that is equal to the natural frequency of the first molar and its periodontal tissue stimulates more effectively the periodontal tissue.[16] Thus, we hypothesized that the application of resonance vibration during orthodontic tooth movement should affect the acceleration of tooth movement by increasing the activity of the cells in the PDL.

Our aims in this study were to investigate the effects of stimulation by resonance vibration on the speed of tooth movement and root resorption during experimental tooth movement in rats, and to elucidate the cellular and molecular mechanisms underlying the acceleration of tooth movement.

MATERIAL AND METHODS

We developed a vibration-imposed system (in conjunction with IMV Corp, Osaka, Japan) that enables us to apply a forced vibration with continuously changing frequency onto the teeth, and to measure the natural frequency of the teeth and periodontium. This system comprises a vibration controller (RC-1120; IMV), a power amplifier (PET-OA; IMV), a charge amplifier (5011B; Kistler Instrumente AG, Winterthur, Switzerland), and a vibrator (PET-01; IMV) (Fig 1). A force sensor (Kistler Instrumente AG) and an accelerometer (Endevco, San Juan Capistrano, Calif) were built into the top of the vibrator. The top of the vibrator was fixed on the rats' first molars with an adhesive (Superbond; Touagousei, Tokyo, Japan). The signals from the force

sensor and the accelerometer were transferred into the vibration controller.

The vibration was applied by the control signal through the power amplifier controlled by the output signal from the accelerometer, thereby maintaining the acceleration at 1.0 meter per square second (m/s^2).

The amplified signal was then transferred to the vibrator, causing its excitation. The change in frequency response of the specimen can be detected simultaneously. The vibration tests were carried out for 5 minutes, and the resonance curves were displayed as frequency-force relationships on the monitor of the vibration controller.

The animals were treated according to the Guidelines for the Use of Experimental Animals of the Animal Care and Use Committee of Tohoku University, Graduate School of Dentistry. Six male Wistar rats (Japan SLC, Shizuoka, Japan) were used for the measurements of resonance frequency in the their first molars. The heads of the rats were fixed on the stereotaxic frame (SR60; Narishige, Tokyo, Japan) under anesthesia from intraperitoneal injection of sodium pentobarbital (50 mg per kilogram of body weight). The excitation rod was fixed to the first molar perpendicular to the occlusal plane with adhesive, and the resonance frequency of the first molar was measured (Fig 2, *A*).

Generally, the relationship between displacement (X) and the ratio of frequency (ie, natural frequency) is shown in Figure 2, *B*. This system encounters a condition of resonance at the maximum amplitude. In this study, because the frequency of forced vibration was controlled at 1.0 m/s^2, and, taking $F = kX$ and $F = ma$ into consideration, the relationship in Figure 2, *B,* can be replaced with the relationship in Figure 2, *C*, where m is the mass of the system, k is the spring constant, X is the maximum displacement, and F is the externally applied force. Consequently, the resonance frequency should be minimal in Figure 2, *C*. Figure 2, *D*, was the resonance curve displaced on the monitor of the resonance vibration delivery system.

In total, 42 male Wistar rats were used; 12 rats for measuring tooth movement and 30 rats for histological examination.

To measure tooth movement, we used 12 male Wistar rats (age, 6 weeks) with an average weight of 150 g. The animals were adapted to a 12/12-hour light/dark cycle (with light from 07:00 to 19:00) for a week in a room at 25°C and 55% humidity. They were given laboratory chow (Funabashi Farms, Funabashi, Japan) and deionized water ad libitum.

The rats were divided into 2 groups of 6 animals each: the resonance vibration group (RV-group), loaded with the resonance frequency, and the control group (C-group). In the RV-group, a uniform standardized expansive spring[17] made of 0.012-in nickel-titanium wire (Nitinol Classic; 3M Unitek Dental Products, Monrovia, Calif) was placed between the maxillary right and left first molars (Fig 3, *A* and *B*), and used to move the teeth in the buccal direction for 21 days. The load-deflection curve of the expansive spring was examined by using a creep meter (RE2-33005S; Yamaden, Tokyo, Japan). The load-deflection curve is shown in Figure 3, *C*. The initial expansive force to each tooth generated by the spring was an average of 12.8 gram force. The wire was retained in the mouth by its own force. Resonance frequency was loaded perpendicularly on the maxillary molars for 8 minutes once a week after removing the expansive spring under general anesthesia with intraperitoneal injection of sodium pentobarbital (50 mg per kilogram of body weight). After loading of resonance frequency, the adhesive was removed from the occlusal surface completely, so that it did not interfere with occlusion.

The C-group had the same procedures except that the machine was not turned on.

Displacement of the maxillary first molars was measured on days 0, 3, 7, 10, 14, 17, and 21, after application of the expansive force. The animals were anesthetized lightly with ether, and precise silicone impressions (G-C Dental Industrial, Tokyo, Japan) with resin trays were used to produce a stone model of each rat's maxillary dentition. The base of each stone model was trimmed to parallel the occlusal plane and then set on the stage of a profile projector (V-16D; Nikon, Tokyo, Japan); it was subsequently magnified 10-fold on a screen with reflected light. A tracing of the occlusal view of a precise plaster model of the maxillary dentition was magnified 10-fold. The contours of the palatal cusps of the second and third molars of these tracings were then superimposed on those traced from the initial plaster model. The distance between the mesiopalatal cusp crest of the first molar before and after tooth movement was measured with sliding calipers. Tooth movement alone, excluding growth of the midpalatal suture, was measured by superimposing the contours of the second and third molars. The values obtained for the right and left first molars were summed for each animal.

Thirty male Wistar rats were used for the histological examinations. Twenty-seven rats were divided into 2 groups, with 13 animals in the RV-group and 14 animals in the C-group. Animals from each group were killed on days 3, 8, and 15 (3 animals, respectively) and on day 21 (4 animals in the RV-group and 5 animals in

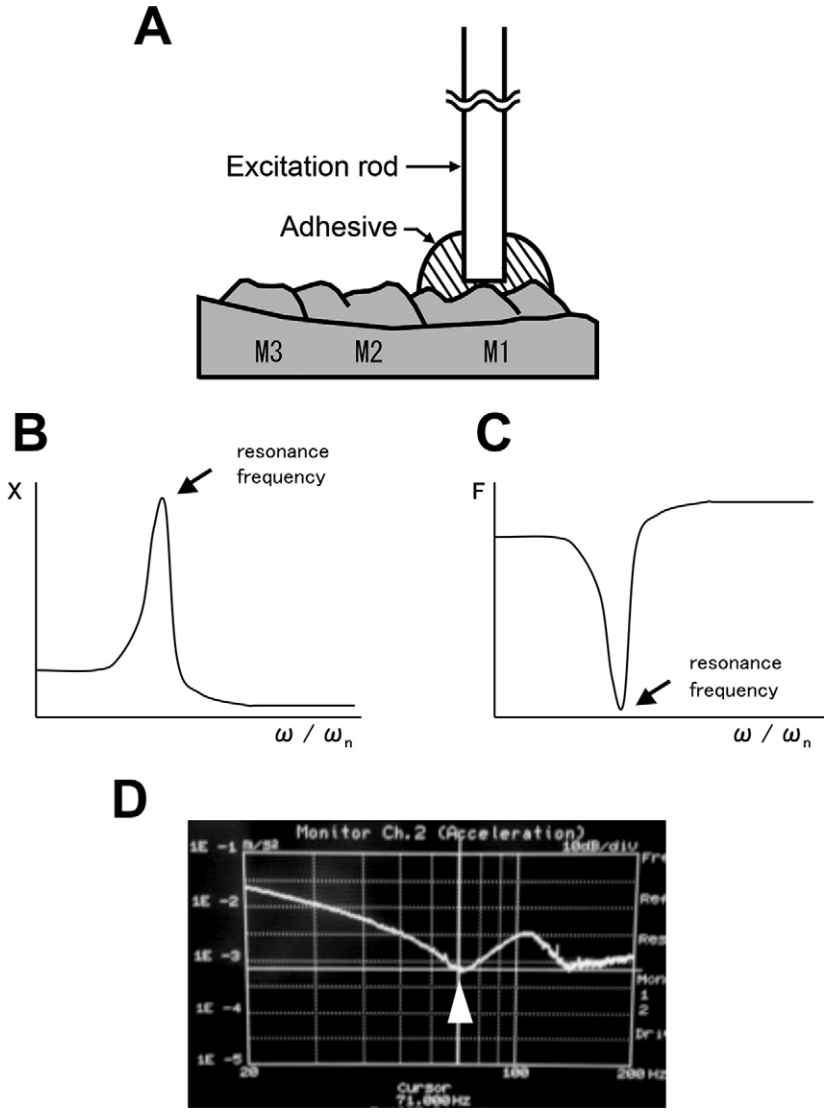

Fig 2. Resonance curve: **A,** schema of buccal section in rat molar. The excitation rod was fixed to the first molar perpendicular to the occlusal plane with adhesive. **B,** The relationship between the displacement (*X*) and the ratio of frequency (ω/ω_n). **C,** The relationship between the exciting force (*F*) and the ratio of frequency (ω/ω_n). **D,** The resonance curve is displaced on the monitor of the resonance vibration delivery system. The horizontal axis shows the frequency (Hz), and the vertical axis shows the force (N). *Arrowhead,* natural frequency; *M1,* first molar; *M2,* second molar; *M3,* third molar.

the C-group), after application of the force. The remaining 3 animals served as the day 0 control.

After the 21-day experiment, the animals were killed by using intraperitoneal injection of sodium pentobarbital (50 mg per kilogram of body weight), and the tissues were fixed by perfusing the animals through the ascending aorta with 4% paraformaldehyde in 0.01 mol/L of phosphate buffer (pH 7.4). The maxillary jaws, including the molars, were dissected, fixed overnight at 4°C, and decalcified in 10% EDTA in PBS. They were then dehydrated in a graded series of ethanol and embedded in paraffin. The embedded specimens were cut into serial 5-μm-thick cross-sections perpendicular to occlusal plane. The sections were stained with hematoxylin and eosin. The mesiopalatal root of the maxillary first molar was evaluated for root resorption.[18] Microscopic images taken directly from a high-resolution monitor with a CCD video camera (Cool-

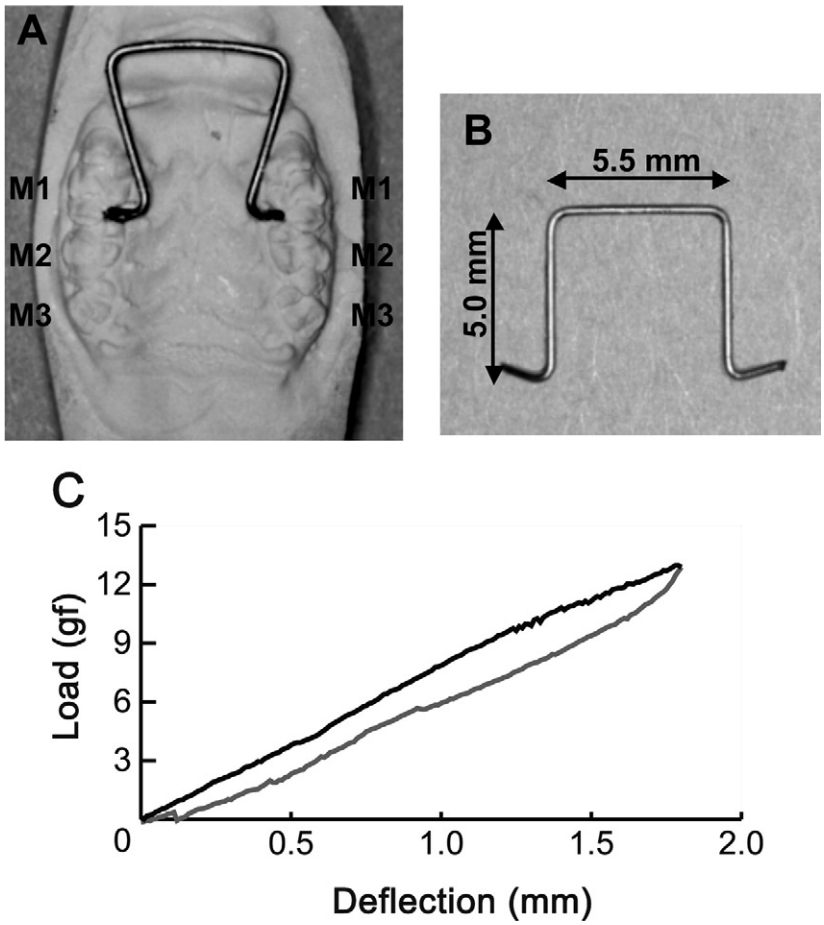

Fig 3. A, Expansive spring placed between the maxillary right and left first molars of the rats; **B,** uniform standardized expansive spring was made of 0.012-in nickel-titanium wire; **C,** load-deflection curve of the expansive spring. *M1,* first molar; *M2,* second molar; *M3,* third molar; *gf,* gram force.

SNAP; Olympus, Tokyo, Japan) were analyzed by Scion-image (Scion, Frederick, Md). The areas of root resorption in the right and left sides of each section were averaged, and the values for 8 sections, selected at 50-μm intervals from the root bifurcation (interradicular region), were averaged for each animal.

The sections were stained for tartrate-resistant acid phosphatase (TRAP) activity using the acid phosphate leukocyte kit (Sigma Chemical, St Louis, Mo), to identify the osteoclasts. The osteoclasts on alveolar bone surfaces or in bone-resorptive lacunae at the pressure side were counted in each section on days 8, 15, and 21. Osteoclasts were recognized as TRAP-positive multi-nucleated cells. The numbers of osteoclasts on the right and left sides of each section were initially averaged. The values obtained for the 8 sections selected at 50-μm intervals from the root interradicular region were subsequently averaged for each animal.

The sections were processed for immunocytohistochemistry. To inhibit the endogenous peroxidase activity, dewaxed sections were treated with 0.3% hydrogen peroxide in methyl alcohol. After blocking nonspecific reactivity by treatment with 10% bovine serum albumin in PBS, the sections were reacted with goat polyclonal antibodies directed against human RANKL (Santa Cruz Biotechnology, Santa Cruz, Calif) at a dilution of 1:100 for 24 hours at 4°C. After washing in PBS, the sections were incubated with peroxidase-conjugated donkey antigoat IgG (sc-2020; Santa Cruz Biotechnology) for 1 hour at room temperature. To visualize immunoreactivity, the sections were flooded with a solution of diaminobenzidine. Counterstaining for light microscopy was carried out with hematoxylin. As a negative

Table. Resonance frequency, velocity, and displacement values of the maxillary first molars of rats

Rat	Resonant frequency (Hz)	Velocity (mm/s)	Displacement (mm)
1	48.50	0.33	0.022
2	51.40	0.31	0.019
3	62.00	0.26	0.013
4	64.40	0.25	0.012
5	71.00	0.22	0.010
6	68.80	0.23	0.011
Average	61.02	0.27	0.014
Standard error	3.75	0.018	0.002

control, 0.5% bovine serum albumin in PBS was used instead of the primary antiserum.

Statistical analyses

The values are represented as the mean ± SEM for each group. The data on tooth movement and body weight were subjected to both analysis of variance (2-way repeated measures ANOVA) and the Student *t* test. The data on osteoclast numbers were analyzed with both the 2-factor factorial ANOVA and the Student *t* test. The data on root-resorptive areas were subjected to the Student *t* test. A *P* value ≤.05 was considered to be statistically significant.

RESULTS

The mean resonance frequency of the rat first molar was 61.02 ± 8.375 Hz (mean ± SEM). The mean displacement in resonance was 0.0014 ± 0.002 mm, and the average velocity was 0.27 ± 0.018 mm per second. The resonance frequency, displacement, and velocity values for each rat are shown in the Table.

The average body weights of the rats increased gradually and linearly in both the RV-group and the C-group. There was no significant difference in body weights between the 2 groups during the experimental period.

Figure 4 shows the time course of tooth movement in the 2 groups of animals. Statistically significant differences in treatment and time were assessed by 2-way repeated measures ANOVA ($P < .05$). Furthermore, the extent of tooth movement on day 21 was significantly greater by 15% in the RV-group than in the C-group ($P = .008$, Student *t* test).

More osteoclasts were found on the alveolar bone surfaces of the RV-group rats than on those of the C-group rats ($P < .08$ for treatment by 2-factor factorial ANOVA) (Fig 5, *A* and *B*). In the C-group, the number of osteoclasts increased gradually, whereas numerous osteoclasts were found on day 8 and persisted until day 21 in the RV-group (Fig 5, *C*). In addition, the number of osteoclasts was significantly higher in the RV-group than in the C-group on day 8 ($P < .05$, Student *t* test).

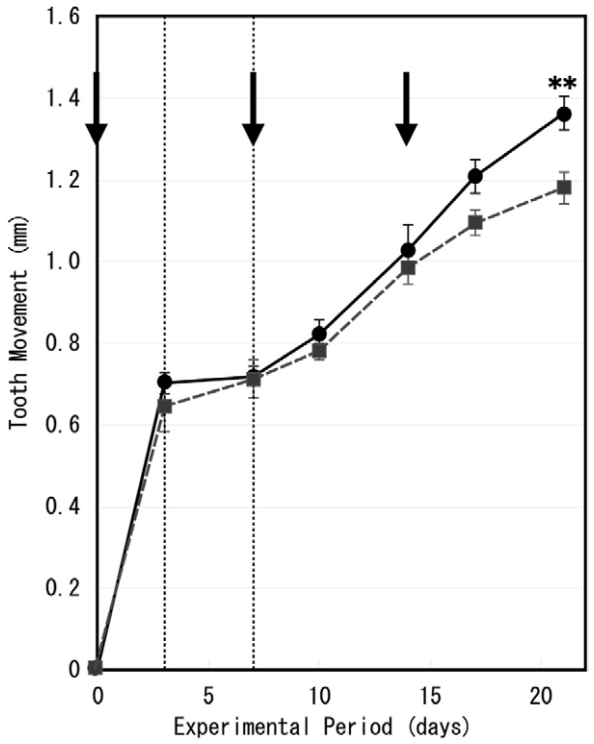

Fig 4. Time course of tooth movement in the RV-group and the C-group. Tooth movement in both groups was divided into 3 phases. The extent of tooth movement in the RV-group was significantly higher than in the C-group; $P < .05$ for treatment and time by 2-way repeated measures ANOVA. $^{**}P < .01$ by the Student *t* test. Each value represents the mean ± SEM (n = 6). *Arrow* indicates the timing of resonance vibration on days 0, 8, and 15. *Line with black circles,* RV-group; *line with gray squares,* C-group

RANKL-positive cells were observed in the PDLs of both the RV-group and the C-group. RANKL immunostaining was observed in the osteoblasts and PDL fibroblasts on the tension side. On the compression side, RANKL immunostaining was observed in PDL fibroblasts and multinucleated osteoclasts. RANKL expression was stronger on the compression side than on the tension side. In particular, on day 3, RANKL expression on the compression side in the RV-group was higher than that on the compression side in the C-group (Fig 6). On days 8, 15, and 21, RANKL immunostaining was observed on both the compression side and the tension side in the RV-group and the C-group. However, there was no significant difference

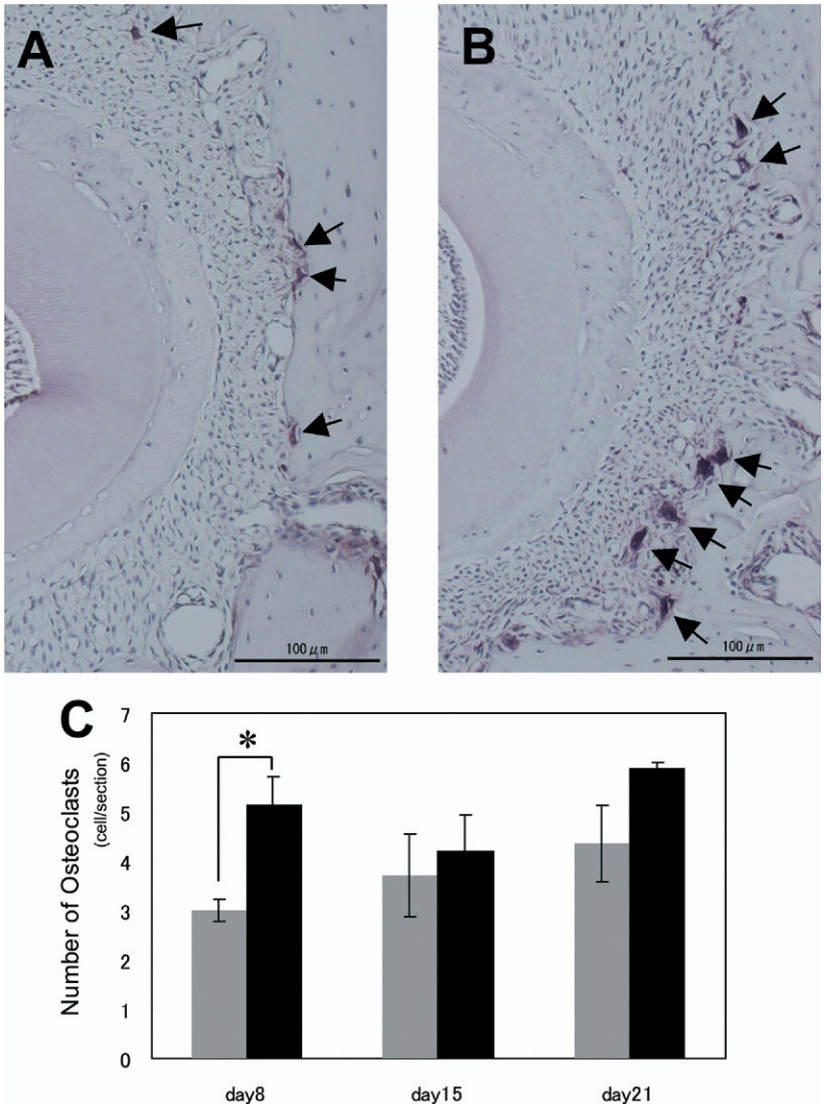

Fig 5. A and **B,** Histologic comparison of osteoclast distribution on day 8 in rat PDLs with or without resonance vibration. TRAP staining was performed. Many TRAP-positive multinucleated cells were observed on the alveolar bone surface in the RV-group (*B*) compared with the C-group (*A*). *Arrows,* TRAP-positive multinucleated cells. **C,** Numbers of osteoclasts at the compressed sides of the rats in the RV-group and the C-group. *Gray,* C-group; *black,* RV-group. $P < .05$ between groups by 2-factor factorial ANOVA. *$P < .05$ by Student *t* test. Each value represents the mean ± SEM (n = 3-5).

in the intensity of RANKL immunostaining between the 2 groups. There was no histologic differences between animals without tooth movement and RV-treated animals without tooth movement.

Root resorption was observed on the root surface on the compression side in both groups. Resorption was observed not only in the cementum but also in the dentin (Fig 7, *A* and *B*). In the quantitative evaluation, there was no significant difference in the level of root resorption between the 2 groups on day 21 (Fig 7, *C*).

DISCUSSION

We stimulated the periodontal tissue by resonance vibration to accelerate the speed of tooth movement and shorten the treatment period. We have clearly demonstrated the stimulatory effects of resonance vibration in accelerating the speed of tooth movement with no

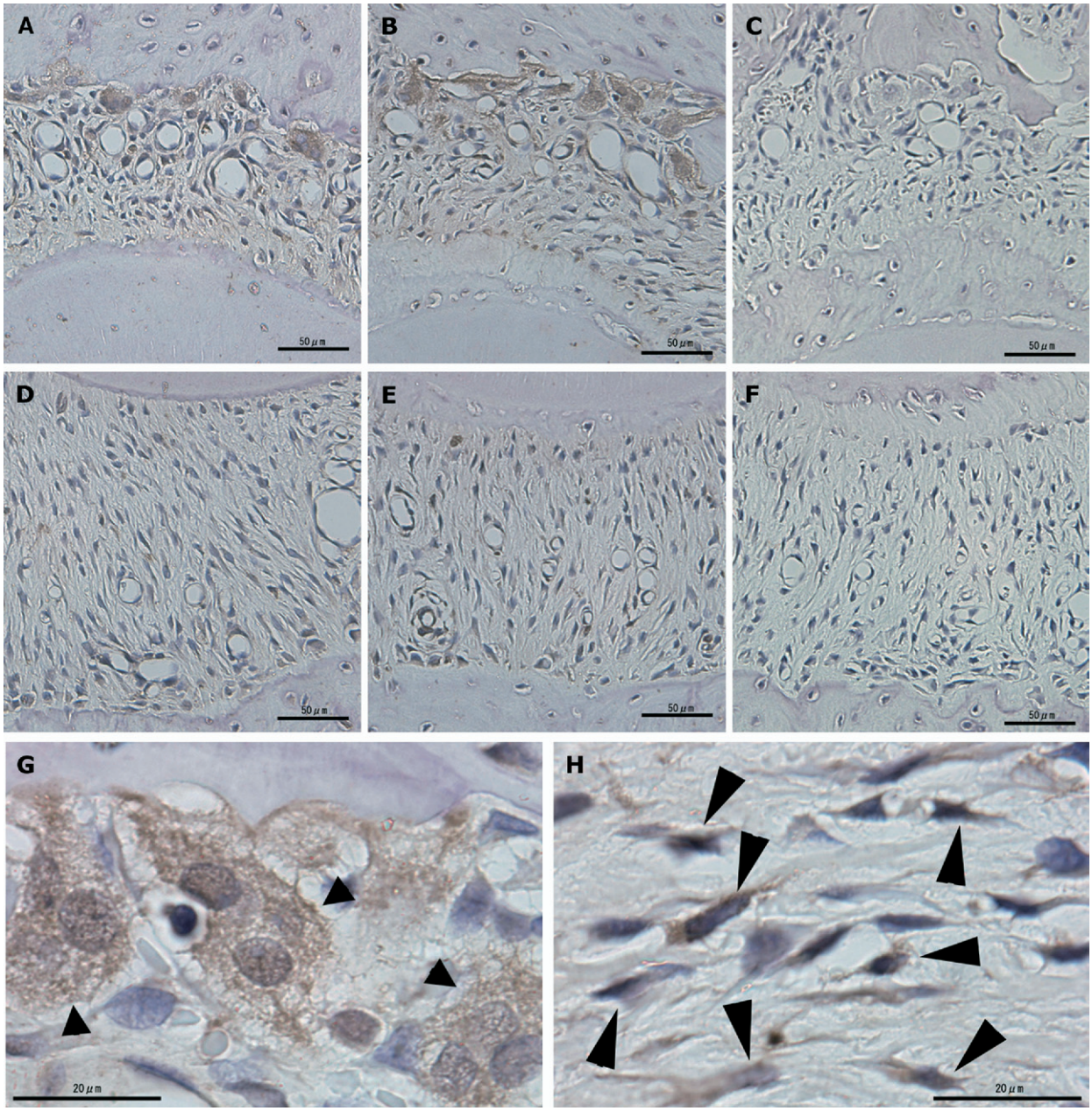

Fig 6. Comparison of RANKL distribution in rat PDLs with or without intermittent stimulation by resonance vibration. Strong RANKL expression was observed on day 3 in the PDL cells of the RV-group. **A,** RANKL immunostaining in the PDL tissue at the compressed side of the C-group on day 3; **B** and **G,** RANKL immunostaining in the PDL tissue at the compressed side of the RV-group on day 3; **C** and **F,** negative control (secondary antibody only); **D,** RANKL immunostaining in the PDL tissue at the tension side of the C-group on day 3; **E** and **H,** RANKL immunostaining in the PDL tissue at the tension side of the RV-group on day 3. *Small arrows,* RANKL immunostaining of osteoclasts; *large arrows,* RANKL immunostaining of PDL fibroblasts

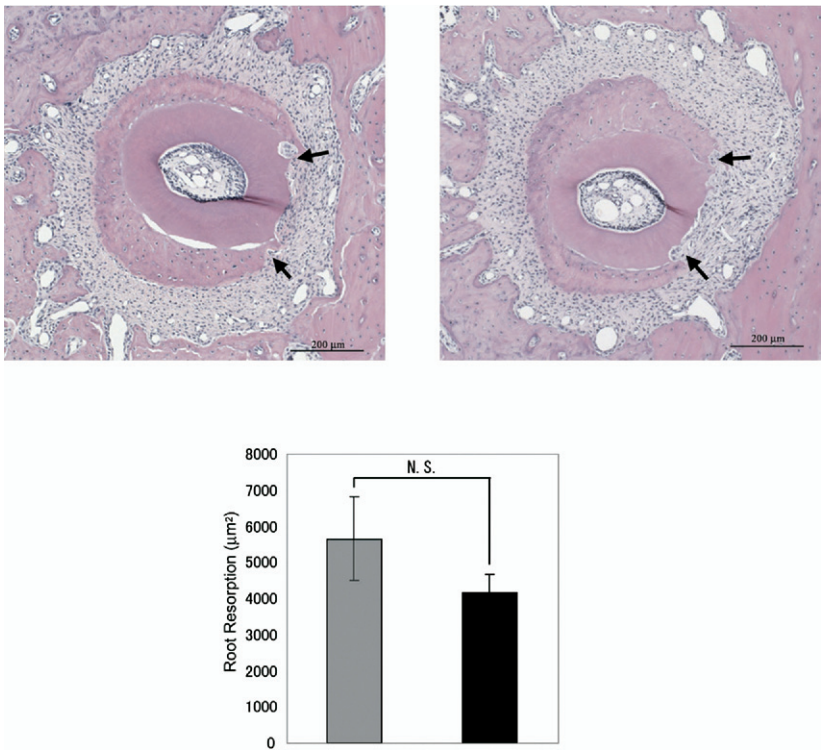

Fig 7. Microscopic observation of the mesiopalatal root of the maxillary first molar in **A,** RV-group and **B,** C-group on day 21. Hematoxylin and eosin staining. Root resorption is observed at the compressed side in both groups. *Arrow,* root resorption. **C,** Area of root resorption in the mesiopalatal root of the maxillary first molar on day 21. Each value represents the mean ± SEM (n = 4-5). *Gray,* C-group; *black,* RV-group. There was no significant difference between the 2 groups.

collateral damage to periodontal tissues. In addition, we have demonstrated the activation of the RANK-RANKL signaling pathway in response to the loading of resonance vibration.

In this study, we loaded the resonance vibration onto the rats' first molars to examine its influence on orthodontic tooth movement. The appliance used to induce tooth movement was simple, and it kept the mouths of the rats clean. During the experiment, the mean body weight of the rats increased gradually and linearly; there was no significant difference between the groups in this respect. The health and growth of the animals were not affected by the anesthesia, nor were they affected by the impression of the maxilla or the application of resonance vibration.

We considered it necessary to measure the preexisting resonance frequency of each rat before loading the resonance vibration. Nevertheless, we loaded all rats with the average resonance frequency, to avoid the potential adverse effects of anesthesia over several hours. We measured the natural frequency of the rat first molar according to the method reported by Kurashima[19] and Kato.[20] The resonance frequency of the tooth was measured by continuously transforming the forced vibration. The resonance frequency—the natural frequency—was defined as the maximum recorded velocity in the resonance curve. The resonance vibration was considered to be the force that applied the largest amplitude of vibration to the periodontal tissue.

It was reported that signaling molecules, such as c-fos,[13] MAPK,[14] and nitric oxide[15] are increased in the PDL immediately after mechanical stimulation. Therefore, we considered it possible to activate PDL cells using an initial short-term stimulation. Stimulation was applied for only 8 minutes. Loading a vibrational force for 1.5 hours per day over 3 weeks was reported to give about 1.3 to 1.4 times greater tooth movement than loading a static force.[21] However, the frequency and duration of this type of treatment entail considerable mental and physical stresses. Therefore, it is desirable in the clinical setting to apply vibrational stimulation as briefly as possible. We examined the effect of vibrational stimulation for the shortest possible period of application required to activate the PDL; this was

determined to be 8 minutes in our pilot study. We used vibrational stimulation in addition to the static force applied by the orthodontic wire.

The vibration was applied once a week. The complex of tooth and PDL is considered to be viscoelastic.[19] It was reported that an intermittent vibrating force is mechanically more effective than a static force in changing the PDL's viscoelasticity, and that this effect persists over a certain period of time.[16] In our study, a significant difference was noticed when the vibration was applied once a week. Nonetheless, future studies should examine the effects of vibration at intervals of longer than a week.

It is generally considered that tooth movement that is due to heavy orthodontic forces occurs in 3 phases.[22-24] Our findings show these aspects of tooth movement. It appears that the speed of tooth movement greatly depends on the speed of alveolar bone remodeling.

The speed of tooth movement was influenced mainly by bone resorption, with osteoclasts induced on the alveolar bone surface on the pressure side. Therefore, we counted the multinuclear osteoclasts on the alveolar bone surfaces during the final phase. In the C-group, the number of multinuclear osteoclasts was significantly low on day 8 and increased in a time-dependent manner. On the other hand, numerous multinuclear osteoclasts were observed in the RV-group from days 8 to 21. This result suggests that differences in the appearance of multinuclear osteoclasts can affect the speed of tooth movement during the final phase.

Recently, RANKL has been reported as an essential factor for osteoclast formation, function, and survival.[25-27] Therefore, we examined RANKL expression in the PDL after resonance vibrational stimulation. Our results indicate that RANKL is expressed in osteoblasts, PDL fibroblasts, and multinucleated osteoclasts. Furthermore, RANKL expression was expressed strongly on the compression side in the RV-group compared with the C-group. It is generally accepted that RANKL is expressed in stromal cells, fibroblasts, and osteoblasts. However, several reports showed that RANKL is expressed in osteoclasts.[28,29] Kartsogiannis et al[28] reported that the levels of RANKL mRNA and protein appear to correlate with resorptive capability, whereby osteoclasts on actively resorbed surfaces display high-level RANKL expression. Our findings suggest that resonance vibration stimulates the resorptive activities of osteoclasts. The number of multinuclear osteoclasts on day 8 was 1.7 times higher in the RV-group than in the C-group. These findings suggest that resonance vibration stimulates the differentiation of monocytes/macrophages from hematopoietic cells by, for example, increasing the blood flow. Moreover, increased RANKL expression in PDL fibroblasts and osteoclasts might induce and activate osteoclasts. Consequently, alveolar bone remodeling could be enhanced.

Noteworthily, resonance vibration can be applied as a mechanical stress on PDL cells. Ultrasonic vibration is a form of vibrational stimulation that is similar to resonance vibration. It has been reported that ultrasonic vibration accelerates tooth movement.[30] However, ultrasonic vibration of teeth might be associated with certain hazards, such as thermal damage to the dental pulp.[31] On the other hand, we believe that the resonance vibration we used in this study is efficient and can be applied to the PDL as a mechanical stress that does not cause additional damage to the periodontal tissues.

Ultrasound is used to treat bone fractures in orthopedics. The effects of ultrasound have been shown in soft tissues and include angiogenesis,[32] increased protein synthesis in fibroblasts,[33] and increased blood flow velocity in the muscular distribution artery.[34] It has also been reported that ultrasound effects bone repair.[35,36] The exact cellular mechanism underlying the therapeutic action of ultrasound remains unknown, although the following hypotheses have been proposed: (1) a direct effect on the permeability of the cell membrane and second messenger adenylate cyclase activity and changes in ion or protein transport, which could modify the intracellular signals for gene expression[37,38]; (2) activation of the "stretch receptor" type of cation channel and changes in the cation concentration, so as to modify the intracellular signals that regulate gene expression[39]; (3) transferred mechanical energy activates changes in the attachment of the cytoskeleton to the extracellular matrix[40]; and (4) the induction of electrical currents in the bone. A rise in temperature might have an effect on cell metabolism.[34] It is speculated that some of the above possible events could be involved in the underlying mechanism in the effect of resonance vibration on tooth movement, however, the detailed mechanism has not been reported until now. Further studies are required to elucidate these phenomena.

We have already mentioned the effect of resonance vibration on tooth movement. It is thought that odontoclasts, which have the same origin as osteoclasts, are induced by the same mechanism. In orthodontic treatment, root resorption by odontoclasts is a form of severe additional damage. We examined whether root resorption was accelerated in the same way as tooth movement was accelerated by resonance vibration. Large lacunae were found in the 2 groups, but no severe resorption, which was observed with forces of higher

magnitude, was seen. So we believe that the force of the 12.8 gram force was the optimal force level to move the rat molars.

We found no significant difference in root resorption between the 2 groups. However, this investigation indicated a trend toward less root resorption in the RV-group. Root resorption during orthodontic tooth movement is generally thought to occur because hyalinized tissue, which results from blood flow obstruction at the compression side, accelerates root resorption.[41-43] Therefore, we hypothesized that resonance vibration might prevent blood flow obstruction and hyalinization at the compression side. Although several similarities have been reported between osteoclasts and odontoclasts,[44] the differences in their differentiation and resorption activities are not clearly known. Our results suggest that resonance vibration affects osteoclasts but not odontoclasts during experimental tooth movement in vivo. Therefore, the mechanism by which resonance vibration reduces root resorption merits further investigation.

CONCLUSIONS

The application of resonance vibration might accelerate orthodontic tooth movement via enhanced RANKL expression in the PDL with no additional damage to periodontal tissues, such as root resorption.

We thank Drs Yoshinobu Shimizu, Tokushi Emata, Naoto Haruyama, Naoya Sakamoto, and Mr Toshihiro Onodera for providing valuable advice.

REFERENCES

1. Segal GR, Schiffman PH, Tuncay OC. Meta analysis of the treatment-related factors of external apical root resorption. Orthod Craniofac Res 2004;7:71-8.
2. Kawasaki K, Shimizu N. Effects of low-energy laser irradiation on bone remodeling during experimental tooth movement in rats. Lasers Surg Med 2000;26:282-91.
3. Tengku BS, Joseph BK, Harbrow D, Taverne AA, Symons AL. Effect of a static magnetic field on orthodontic tooth movement in the rat. Eur J Orthod 2000;22:475-87.
4. Stark TM, Sinclair PM. Effect of pulsed electromagnetic fields on orthodontic tooth movement. Am J Orthod Dentofacial Orthop 1987;91:91-104.
5. Yamasaki K, Miura F, Suda T. Prostaglandin as a mediator of bone resorption induced by experimental tooth movement in rats. J Dent Res 1980;59:1635-42.
6. Yamasaki K, Shibata Y, Imai S, Tani Y, Shibasaki Y, Fukuhara T. Clinical application of prostaglandin E_1 (PGE_1) upon orthodontic tooth movement. Am J Orthod 1984;85:508-18.
7. Leiker BJ, Nanda RS, Currier GF, Howes RI, Sinha PK. The effects of exogenous prostaglandins on orthodontic tooth movement in rats. Am J Orthod Dentofacial Orthop 1995;108:380-8.
8. Collins MK, Sinclair PM. The local use of vitamin D to increase the rate of orthodontic tooth movement. Am J Orthod Dentofacial Orthop 1988;94:278-84.
9. Takano-Yamamoto T, Kawakami M, Yamashiro T. Effect of age on the rate of tooth movement in combination with local use of $1,25(OH)_2D_3$ and mechanical force in the rat. J Dent Res 1992;71:1487-92.
10. Takano-Yamamoto T, Kawakami M, Kobayashi Y, Yamashiro T, Sakuda M. The effect of local application of 1,25-dihydroxycholecalciferol on osteoclast numbers in orthodontically treated rats. J Dent Res 1992;71:53-9.
11. Brudvik P, Rygh P. Root resorption after local injection of prostaglandin E_2 during experimental tooth movement. Eur J Orthod 1991;13:255-63.
12. Ochiai H. Histological investigations on the effect of prostaglandin E2 (PGE2) applied to experimental tooth movement. Nippon Kyosei Shika Gakkai Zasshi 1987;46:500-16.
13. Yamaguchi N, Chiba M, Mitani H. The induction of c-fos mRNA expression by mechanical stress in human periodontal ligament cells. Arch Oral Biol 2002;47:465-71.
14. Matsuda N, Morita N, Matsuda K, Watanabe M. Proliferation and differentiation of human osteoblastic cells associated with differential activation of MAP kinases in response to epidermal growth factor, hypoxia, and mechanical stress in vitro. Biochem Biophys Res Commun 1998;249:350-4.
15. Kikuiri T, Hasegawa T, Yoshimura Y, Shirakawa T, Oguchi H. Cyclic tension force activates nitric oxide production in cultured human periodontal ligament cells. J Periodontol 2000;71:533-9.
16. Emata T. The mechanical response of the periodontal structure in the maxillary lateral incisor of the macaca fuscata yakui, loading by a vibrating force. J Oral Biol Sci 1979;21:571-85.
17. Igarashi K, Mitani H, Adachi H, Shinoda H. Anchorage and retentive effects of a bisphosphonate (AHBuBP) on tooth movement in rats. Am J Orthod Dentofacial Orthop 1994;106:279-89.
18. Miyoshi K, Igarashi K, Saeki S, Shinoda H, Mitani H. Tooth movement and changes in periodontal tissue in response to orthodontic force in rats vary depending on the time of day the force is applied. Eur J Orthod 2001;23:329-38.
19. Kurashima K. The viscoelastic properties of the periodontal membrane and alveolar bone. Kokubyo Gakkai Zasshi 1963;30:361-85.
20. Kato H. Measurement of resonance frequency of normal and pathological periodontium. New method of periodontal examination. Kokubyo Gakkai Zasshi 1968;38:58-81.
21. Shimizu Y. A study of the movement of the lateral incisor of the macaca fuscata loaded by a vibrating force. Nippon Kyosei Shika Gakkai Zasshi 1986;45:56-72.
22. Storey E. The nature of tooth movement. Am J Orthod 1973;63:292-314.
23. Reitan K. Clinical and histologic observations on tooth movement during and after orthodontic treatment. Am J Orthod 1967;53:721-45.
24. Burstone CJ, Tanne K. Biomechanical basis of tooth movement. Kokubyo Gakkai Zasshi 1986;45:541-51.
25. Yasuda H, Shima N, Nakagawa N, Yamaguchi K, Kinosaki M, Mochizuki S, et al. Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL. Proc Natl Acad Sci 1998;95:3597-602.
26. Lacey DL, Timms E, Tan HL, Kelley MJ, Dunstan CR, Burgess T, et al. Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. Cell 1998;93:165-76.
27. Boyle WJ, Simonet WS, Lacey DL. Osteoclast differentiation and activation. Nature 2003;423:337-42.
28. Kartsogiannis V, Zhou H, Horwood NJ, Thomas RJ, Hards DK, Quinn JM, et al. Localization of RANKL (receptor activator of

NF kappa B ligand) mRNA and protein in skeletal and extraskeletal tissues. Bone 1999;25:525-34.
29. Shiotani A, Shibasaki Y, Sasaki T. Localization of receptor activator of NFkappaB ligand, RANKL, in periodontal tissues during experimental movement of rat molars. J Electron Microsc (Tokyo) 2001;50:365-9.
30. Ohmae M, Saito S, Morohashi T, Qu H, Seki K, Kurabayashi H, et al. Biomechanical acceleration of experimental tooth movement by ultrasonic vibration in vivo—part 1. Homo-directional application of ultrasonication to orthodontic force. Orthod Waves 2001;60:201-12.
31. Trenter SC, Walmsley AD. Ultrasonic dental scaler: associated hazards. J Clin Periodontol 2003;30:95-101.
32. Young SR, Dyson M. The effect of therapeutic ultrasound on angiogenesis. Ultrasound Med Biol 1990;16:261-9.
33. Doan N, Reher P, Meghji S, Harris M. In vitro effects of therapeutic ultrasound on cell proliferation, protein synthesis, and cytokine production by human fibroblasts, osteoblasts, and monocytes. J Oral Maxillofac Surg 1999;57:409-19.
34. Fabrizio PA, Schmidt JA, Clemente FR, Lankiewicz LA, Levine ZA. Acute effects of therapeutic ultrasound delivered at varying parameters on the blood flow velocity in a muscular distribution artery. J Orthop Sports Phys Ther 1996;24:294-302.
35. Reher P, Elbeshir el-NI, Harvey W, Meghji S, Harris M. The stimulation of bone formation in vitro by therapeutic ultrasound. Ultrasound Med Biol 1997;23:1251-8.
36. Azuma Y, Ito M, Harada Y, Takagi H, Ohta T, Jingushi S. Low-intensity pulsed ultrasound accelerates rat femoral fracture healing by acting on the various cellular reactions in the fracture callus. J Bone Miner Res 2001;16:671-80.
37. Dyson M. Non-thermal cellular effects of ultrasound. Br J Cancer Suppl 1982;45: 165-71.
38. Mortimer AJ, Dyson M. The effect of therapeutic ultrasound on calcium uptake in fibroblasts. Ultrasound Med Biol 1988;14:499-506.
39. Sachs F. Mechanical transduction by membrane ion channels: a mini review. Mol Cell Biochem 1991;104:57-60.
40. Wang N, Butler JP, Ingber DE. Mechanotransduction across the cell surface and through the cytoskeleton. Science 1993;260: 1124-7.
41. Brudvik P, Rygh P. Root resorption beneath the main hyalinized zone. Eur J Orthod 1994;16:249-63.
42. Brudvik P, Rygh P. The initial phase of orthodontic root resorption incident to local compression of the periodontal ligament. Eur J Orthod 1993;15:249-63.
43. Rygh P. Orthodontic root resorption studied by electron microscopy. Angle Orthod 1977;47:1-16.
44. Sasaki T. Differentiation and functions of osteoclasts and odontoclasts in mineralized tissue resorption. Microsc Res Tech 2003;61:483-95.