Caspase-mediated Apoptosis by Compressive Force Induces RANKL in Cementoblasts

Masaru Yamaguchi, Yukari Minato, Mami Shimizu, Jun Kikuta, Takuji Hikida, and Kazutaka Kasai

Department of Orthodontics, Nihon University School of Dentistry at Matsudo, Matsudo, Chiba 271–8587, Japan

Abstract

Orthodontic root resorption (ORR) is one of the serious adverse events related to orthodontic treatment. Caspases are important effector molecules that mediate the process of apoptosis. However, the relationship between the mechanism underlying ORR and apoptosis in the cementum has not been clarified. In this study, human cementoblast-like cells (HCEMs) were cultured and subjected to pressure conditions assumed during orthodontic tooth movement in vitro. We then examined the effect of compressive force on caspase 3, caspase 8, receptor activator of nuclear factor κB ligand (RANKL) and osteoprotegerin (OPG) release. Furthermore, the association between root resorption and caspase was examined using z-VAD-fmk, a caspase inhibitor. In addition, we used HCEMs to examine the expression of caspase 3, caspase 8, RANKL and OPG under heavy force (HF) or optimum force (OF) in vitro. We then assessed the effects of HF with caspase inhibition using z-VAD-fmk [heavy force + inhibition (HI) group] on RANKL release from HCEMs. The application of HF induced higher levels of caspase 3 and 8 than OF. RANKL expression in cementoblasts was observed after the release of caspase 3 and 8 in the HF group. RANKL expression was significantly decreased protein production was suppressed in the HI group compared with the HF group \((p < 0.05)\). The RANKL/OPG ratio was significantly decreased to about 57% at 9 h and 40% at 12 h in the HI group compared with the HF group \((p < 0.05)\). These findings suggest that cementoblasts produce caspase 3 and 8 under HF conditions to activate the apoptotic pathway and then induce osteoclasts via RANKL. We considered that it as part of the mechanism involved in ORR.

Keywords: apoptosis, caspase, RANKL, HCEMs

Introduction

One of the adverse events in orthodontic tooth movement (OTM) is orthodontic root resorption (ORR). ORR can cause a permanent loss of the root structure. In an epidemiological study by Kaley and Phillips (1), most patients undergoing orthodontic treatment showed shortening of the roots, 3% showed severe root resorption (shortening of > 25% of the root length) at maxillary central incisors. In past research, the type of orthodontic appliance (2), magnitude of the force, duration for which force was applied (3–5), type of tooth movement (reciprocating motion of teeth during orthodontic treatment, so-called “jigging”) (6), have been reported to be related to the severity of root resorption. The application of optimum orthodontic forces causes alveolar bone resorption, and application of heavy orthodontic forces is thought to induce ischemia at the periodontal ligament (PDL), tissue healing and cell death.

Apoptosis is the process of programmed cell death. Many components are required for its execution. Recent studies have suggested that osteocytes of alveolar bone adjacent to the hyaline-treated PDL cause apoptotic cell death during exercise in experiments using rat teeth (7). Apoptosis peaks at 3 days after the application of orthodontic forces (8), and factors influencing cyclin and/or cyclin-dependent kinases can cause cell cycle arrest. In addition, if the cycle continues while the cells’ damaged DNA remains intact, then an apoptotic mechanism is triggered and cells undergo apoptosis (9).

Caspases are a family of cysteine proteases that cleave
proteins after aspartic acid residues and are a major cause of apoptotic cell death. Caspases play a key role in apoptosis signaling pathways as a trigger of cell death, as regulatory elements of the process of cell death and as a function of cell death itself. The initiator (apical) caspases are caspase 2, 8, 9 and 10 and the effector (performer) caspases are caspase 3, 6 and 7. Caspase activation leads to characteristic morphological changes in cells, e.g., contraction, DNA fragmentation, chromatin condensation and the development of plasma membrane vesicles, leading to apoptosis (10). In the present study, we focused on caspase 3 and 8. The cementum, which covers the root surface, is composed of thin calcified tissue produced by cementoblasts. It was recently reported that human primary cementoblasts undergo apoptosis under pressure in vitro (11). However, the relationship between root resorption and apoptosis during orthodontic exercise in cementoblasts has not yet been clarified. We previously reported that receptor activator of nuclear factor κ-B ligand (RANKL) is detected in resorbed roots and PDL tissues exposed to an excessive orthodontic force (12). Hatai et al. (13) suggested that apoptosis occurs on the alveolar bone resorption compression side during OTM. Therefore, apoptosis and RANKL expression may affect the incidence of ORR during OTM. Here, we examined the effects of heavy force (HF) and optimum force (OF) on caspase 3, caspase 8, RANKL and osteoprotegerin (OPG) production using human cementoblasts in vitro. In addition, in order to investigate the relationship between caspase and RANKL, RANKL expression was evaluated following caspase inhibition using benzylxoycarbonyl-Val-Ala-Asp(OMe) fluoromethylketone (z-VAD-fmk), a caspase inhibitor, under heavy compression force.

**Materials and Methods**

**Culture of human cementoblast-like cells (HCEMs)**

Previously established HCEMs (14) were maintained in alpha minimum essential medium (α-MEM; Wako, Osaka, Japan) supplemented with 100 μg/ml of penicillin G (Sigma Chemical Co., St. Louis, MO, USA), 50 μg/ml of gentamicin sulfate (Sigma Chemical Co.), 0.3 μg/ml of amphotericin B (Flow Laboratories, McLean, VA, USA) and 10% fetal calf serum (FCS) (Cell Culture Laboratories, OH, USA). The culture solution was stored at 37°C in a humidified incubator (Forma CO2 incubator MIP-3326; Sanyo Electric Medical System Co., Tokyo, Japan) in the presence of 95% air and 5% CO2.

**Application of compression force**

To reproduce the pressure conditions in OTM, we followed the method of Yamaguchi et al. (15). HCEMs were continuously compressed in a pressure side model of the orthodontic exercise site using a uniform compression system. First, cells were seeded in 100-mm cell culture dishes and cultured in α-MEM containing 10% FCS in the presence of 5% CO2 at 37°C. After overnight incubation, the medium of almost confluent cells was changed to α-MEM containing 1% FCS, the cell layer was placed in a dish and a glass plate was placed on top. Thereafter, the cells were subjected to a compressive force (CF) of 1.0 g/cm² (OF group) or 4.0 g/cm² (HF group) by lead weights at 1, 3, 6, 9, 12, 24 or 48 h. Cells in the control group were treated with a thin glass plate without lead weight producing a CF of 0.032 g/cm² (Fig. 1).

**Addition of caspase inhibitor**

According to the method reported by Rodriguez-Enfedaque et al. (16), z-VAD-fmk (Z-Val-Ala-Asp-fluoromethylketone, Bachem) was added to the medium at a concentration of 50 μM in order to inhibit caspase 3 and 8 before the application of CF. The following groups were evaluated: control + inhibition (CI) group (CF: 0.032 g/cm²) and heavy force + inhibition (HI) group (CF: 4.0 g/cm²).

**Real-time polymerase chain reaction (PCR)**

Total RNA was extracted from HCEMs using the RNeasy Mini kit (Qiagen Co., Tokyo, Japan) and real-time PCR was performed on aliquots containing equal amounts of mRNA. The Prime Script RT Reagent Kit (Takara Co., Shiga, Japan) was used to reverse transcribe mRNA into cDNA according to the manufacturer’s protocol. Real-time PCR amplification was carried out using SYBR Premix Ex Taq (Takara Co.) in a thermal cycler (TP-800 Thermal Cycler Dice; Takara Co.). PCR primers for caspase 3, caspase 8, RANKL, OPG and GAPDH were purchased from Takara Co. and designed with reference to the respective cDNA sequences (Table 1). mRNA levels were then determined using real-time PCR after 1, 3, 6, 9, 12 and 24 h.

**Enzyme-linked immunosorbent assay (ELISA)**

The culture medium was collected of RANKL (sRANKL), caspase 3 and caspase 8 after 1, 3, 6, 9, 12, 24 and 48 h for the ELISA, and the samples were concentrated in Mammalian Protein Extraction Buffer. The sRANKL ELISA kit
Precultured human cementoblast-like cells (HCEMs) were continuously compressed at different weights [4.0 g/cm² in the heavy force (HF) group or 1.0 g/cm² in the optimum force (OF) group]. Briefly, cells were grown in wells to confluence. A glass plate and lead weights were then placed on the confluent cell layer in each well. The number of weights determined the amount of CF. The effects of HF and OF on caspase 3, caspase 8, receptor activator of nuclear factor κ-B ligand (RANKL) and osteoprotegerin (OPG) production were investigated. We then assessed the effects of caspase inhibition using z-VAD-fmk (or its absence) on RANKL mRNA expression in HCEMs.

Table 1. PCR primers for caspase 3, caspase 8, RANKL, OPG and GAPDH were designed with reference to the respective cDNA sequences.

| Caspase 3 | Fw: 5'-AAGCAGAGCCATGGACCAC-3' |
|-----------|---------------------------------|
|           | Rv: 5'-CTGGCAGCATCATCCACACATAC-3' |
| Caspase 8 | Fw: 5'-ATGAGCTGGCTGAAGCAAAC-3' |
|           | Rv: 5'-AAGACCTCAATTCTGATCTGTCAC-3' |
| RANKL     | Fw: 5'-TGGATGCTGAATAATAAGCAGGA-3' |
|           | Rv: 5'-AATTTGCGGCACTTTGGA-3' |
| OPG       | Fw: 5'-AGCTGCATGCTCAAGCAGGA-3' |
|           | Rv: 5'-TTTGCAAACTGATTTCTGG-3' |
| GAPDH     | Fw: 5'-GCACCGTGCAAGCTTGAAC-3' |
|           | Rv: 5'-TGGTGAAGACGCCATTGG-3' |

(Biomedica Medizinprodukte GmbH & Co KG, Wien, Austria), human caspase 3 ELISA kit (R&D Systems, MN, USA) and human caspase 8 ELISA kit (Abcam, Cambridge, UK) were performed according to the manufacturers’ protocols.

Statistical analyses

Each value represents the mean ± standard deviation (SD) of each group. Group means were compared using the Mann-Whitney U test. P values < 0.05 and < 0.01 were considered statistically significant.
Results

Effects of CF on caspase 3, caspase 8, RANKL and OPG mRNA expression levels in HCEMs

Caspase 3 and 8 mRNA expression levels increased more in the HF group than in the OF or control group. Caspase 3 and 8 mRNA expression levels in the HF group increased up to 6 h. In particular, the increase from 3 to 6 h was significantly higher than that at other intervals \((p < 0.01)\) (Fig. 2A, B). Furthermore, RANKL mRNA expression increased significantly more in the HF group than in the OF or control group \((p < 0.01)\). In particular, the values at 6, 9, 12 and 24 h were significantly higher than those at other time points. After RANKL mRNA expression increased peaking at 12 h, it was decreased (Fig. 2C). OPG mRNA expression was lower in the HF group than in the control group, however, this difference was not significant (Fig. 2D). Moreover, the RANKL/OPG ratio was significantly higher in the HF group than in the OF group at 6 \((p < 0.05)\), 9, 12 and 24 h \((p < 0.01)\) (Fig. 2E). In the control group, caspase 3, caspase 8, RANKL and OPG mRNA expression levels were no significant difference during the CF application period (Fig. 2A–D).

Effects of caspase inhibitor on caspase 3, caspase 8, RANKL and OPG mRNA expression levels in HCEMs

In the inhibition experiment, caspase 3 and 8 mRNA expression levels were significantly lower in the HI group than at most time points in the HF group \((p < 0.01)\) (Fig. 3A, B). The HI group demonstrated similar trends as observed in the control group; namely, caspase 3, caspase 8, RANKL and OPG mRNA expression levels were no significant difference during the CF application period. Although RANKL mRNA expression showed an upward trend, it was significantly lower in the HI group than in the HF group at 9 \((p < 0.05)\), 12 and 24 h \((p < 0.01)\) (Fig. 3C). OPG mRNA expression similarly showed a decreasing trend in the HF
and HI groups, however, there was no significant difference from the control group (Fig. 3D). In addition, the RANKL mRNA expression generally showed an upward trend, it was significantly lower in the HI group than that in the HF group. OPG mRNA expression showed a similar decreasing tendency in both HF and HI groups, however, there was no significant difference from the control group. The RANKL/OPG ratio was significantly lower in the HI group than in the HF group. *: $P < 0.05$, **: $P < 0.01$, significantly different from the corresponding HF group. Data are expressed as the means ± SD of four independent experiments.

![Graphs showing Caspase 3 and Caspase 8 activity](image)

**Fig. 3.** Caspase 3, caspase 8, RANKL and OPG mRNA expression levels in each group in the inhibition experiment. In the inhibition experiment, caspase 3 and 8 mRNA expression levels were significantly lower in the HI group than that in the HF group at most time points. Although RANKL mRNA expression generally showed an upward trend, it was significantly lower in the HI group than that in the HF group. OPG mRNA expression showed an increasing trend in both HF and HI groups, however, there was no significant difference from the control group. The RANKL/OPG ratio was significantly lower in the HI group than in the HF group. *: $P < 0.05$, **: $P < 0.01$, significantly different from the corresponding HF group. Data are expressed as the means ± SD of four independent experiments.

**Effects of CF on the release of caspase 3, caspase 8 and sRANKL by HCEMs**

The release of caspase 3, caspase 8 and sRANKL proteins from HCEMs in the OF and HF groups was measured using ELISAs. Caspase 3 and 8 protein concentrations in the HF group increased until 0 to 12 h after that it was decreased (Fig. 4A, B). In contrast, RANKL protein levels significantly increased, peaked at 24 h and subsequently decreased (Fig. 4C). Caspase 3 and 8 protein concentrations were significantly higher in the HF group than in the OF or control group (Fig. 4A, B). ($P < 0.05$ and $P < 0.01$) In contrast, RANKL protein levels showed an upward trend, however, concentrations in the HI group were significantly lower at 12–24 h than those in the HF group (Fig. 4D) ($P < 0.01$).

**Discussion**

We hypothesized that apoptosis mediated by caspase 3 and 8 aggravates ORR by activating RANKL-secreting osteoclasts. In a recent study, Matsuzawa et al. (17) demonstrated the involvement of apoptotic cell death of cementocytes in root resorption during OTM *in vivo*. However, cementoblasts covering the surface layer of the cementum were small, polygonal and fibroblast-like, and morphologically difficult to distinguish from other cells of the PDL. Therefore, in this study, we attempted to clarify the
response of cementoblasts using only HCEMs in vitro. However, there are many uncertainties about the relationship between apoptosis and root resorption of cementoblasts under CF in vitro.

Apoptosis consists of two main pathways: endogenous (mitochondrial) and exogenous (receptor-related). Caspases are common to both pathways. Caspase 8 is involved in the extrinsic pathway and is activated through Fas-Fas ligand signaling. In contrast, caspase 9 is involved in the intrinsic pathway. It is activated by changes in oxidative phosphorylation, hypoxia and oxidative stress (18). And Caspase 3 is one of those included in the effector (performer) caspases (10).

In this study, we examined the effects of HF and OF on caspase 3, caspase 8, RANKL and OPG production using HCEMs. Caspase 3 and 8 mRNA expression was remarkably higher in the HF group than in the OF or control group, peaking at 6 h (Fig. 2A, B). In addition, caspase 3 and 8 protein release was markedly higher in the HF group than in the OF or control group, peaking at 12 h (Fig. 4A, B). These results suggested that caspase levels are increased under HF than under OF in HCEMs.

Diercke et al. (11) reported that the compression of cementoblasts increased caspase expression. Using a gene ontology analysis, they showed that the compression of cementoblasts upregulates genes associated with apoptosis. The expression of apoptosis-related genes (pro- and anti-apoptotic genes) in cementoblasts suggests the participation of apoptosis in the regulation of cementoblasts during OTM. Our findings are in line with the results of this previous study.

RANKL is a factor that induces the production of osteoclasts/odontoclasts by RANK activation, whereas OPG inhibits osteoclastogenesis by competing with RANK for...
binding to RANKL. Thus, RANKL/OPG regulate bone resorption by exerting positive-negative control of RANK activation on osteoclasts (19). The RANKL/OPG ratio is used as an indicator of bone resorption. If RANKL is increased, then bone resorption occurs; conversely, if OPG is increased, then bone formation dominates. It was reported that CF induces the production of RANKL in the PDL in an intensity-dependent manner. Yamaguchi et al. (15) reported that the increase in sRANKL and decrease in OPG were greater in the severe root resorption group than in the non-resorption group of PDL cells. In the present study, we also focused on RANKL and OPG expression in HCEMs. We found that RANKL mRNA expression was significantly higher in the HF group than in the OF or control group, peaking at 12 h (Fig. 2C). The release of sRANKL peaked at 24 h and was also significantly higher in the HF group than in the OF or control group (Fig. 4C). OPG mRNA expression was not significantly different among the groups analyzed, however, a decreasing trend was observed in the HF group compared with the control group (Fig. 2D). Furthermore, the RANKL/OPG ratio was significantly higher in the HF group than in the OF group at 6, 9, 12 and 24 h (Fig. 2E). These results indicate that root resorption is increased in the HF group and RANKL may also be found in cementoblasts. As in previous studies, these results suggest that the increase in root resorption factor RANKL is related to the strength of orthodontic forces in cementoblasts.

We also detected a difference in the peak expression of caspases and RANKL by conducting measurements over time. The release of RANKL in cementoblasts is thought to occur after the release of caspase 3 and 8 under HF conditions. Szymczyk et al. (20) reported that caspase 3 activity is required for RANKL-induced osteoclast differentiation. In their studies, pro-caspase 3 knockout mice had decreased number of osteoclasts in long bones, and RANKL did not promote the differentiation of isolated prechondrocytes. Furthermore, neither wild-type nor RAW 264.7 cells expressed TRAP, and they did not multinucleate following treatment with caspase 3 inhibitors. The results of that study suggest that caspase 3 activity is essential for RANKL-induced osteoclast/odontoclast differentiation for root resorption. Our results suggested that caspase levels peak earlier than RANKL levels, supporting these previous findings. Taken together, caspase-mediated apoptosis is likely related to induction of RANKL.

In addition, in order to investigate the relationship between caspase and RANKL, RANKL expression was evaluated using the caspase inhibitor z-VAD-fmk under HF conditions (HI group). z-VAD-fmk is the most commonly used cell permeable caspase inhibitor, and in most cells, z-VAD-fmk prevents apoptosis. In our study, caspase 3 and 8 mRNA expression was significantly lower in the HI group than in the HF group and did not change during the CF application period (Fig. 3A, B). This finding indicates that caspase is inhibited by z-VAD-fmk. In addition, RANKL mRNA and protein expression were significantly lower in the HI group than in the HF group (Fig. 3C). Furthermore, the RANKL/OPG ratio in the HI group decreased by about 57% at 9 h and 40% at 12 h compared with the HF group, showing a statistically significant difference. We considered that RANKL expression induced by caspase 3 and 8 was decreased by z-VAD-fmk. However, RANKL mRNA expression at 6–12 h and sRANKL protein expression at 9–24 h showed an upward trend in the HI group (Figs. 3C, 4C). These findings suggest that root resorption is caused not only by apoptosis but also by other factors. Recent studies in this vein are investigating the involvement of TNF-α and Notch signaling in exacerbation of root resorption, which are also suggested to increase RANKL (21, 22).

Cementoblasts are known to be involved in the repair of root resorption defects, and it is believed that cementoblast death may contribute to root resorption due to the loss of this repair activity (11). Thus, the repair of root resorption after OTM may fail due to apoptosis of cementoblasts. An impaired function of cementoblasts due to increased apoptosis may also result in more pronounced root resorption.

ORR is an unpredictable complication of orthodontic treatment. The present study showed that cementoblasts are involved in the incidence of ORR under CF. Suppressing apoptosis appears to protect the cementum from ORR. Further research is needed to develop orthodontic treatments without ORR.

**Acknowledgments**

This research was supported in part by Grants-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (Nos. 16K11795, 16K11796, 16K20656, 17K17339 and 15H00648). We wish to thank Professor Takashi Takata and the members at Hiroshima University Graduate School for providing the HCEM cell line.
Conflict of Interest
The authors do not declare conflicts of interest in association with this study.

References
1. Kaley J, Phillips C. Factors related to root resorption in edgewise practice. Angle Orthod, 61: 125–132, 1991.
2. Linge BO, Linge L. Apical root resorption in upper anterior teeth. Eur J Orthod, 5: 173–183, 1983.
3. Owman-Moll P, Kurol J, Lundgren D. The effects of a four-fold increased orthodontic force magnitude on tooth movement and root resorptions. An intraindividual study in adolescents. Eur J Orthod, 18: 287–294, 1996.
4. Owman-Moll P, Kurol J, Lundgren D. Effects of a doubled orthodontic force magnitude on tooth movement and root resorptions. An inter-individual study in adolescents. Eur J Orthod, 18: 141–150, 1996.
5. Chan E, Darendeliler MA. Physical properties of root cementum: Part 5. Volumetric analysis of root resorption craters after application of light and heavy orthodontic forces. Am J Orthod Dentofacial Orthop, 127: 186–195, 2005.
6. Hikada T, Yamaguchi M, Shimizu M, Kikuta J, Yoshino T, Kasai K. Comparisons of orthodontic root resorption under heavy and jiggling reciprocating forces during experimental tooth movement in a rat model. Korean J Orthod, 46: 228–241, 2016.
7. Hamaya M, Mizoguchi I, Sakakura Y, Yajima T, Abiko Y. Cell death of osteocytes occurs in rat alveolar bone during experimental tooth movement. Calcif Tissue Int, 70: 117–126, 2002.
8. Rana MW, Pothisiri V, Killiany DM, Xu XM. Detection of apoptosis during orthodontic tooth movement in rats. Am J Orthod Dentofacial Orthop, 119: 516–521, 2001.
9. Wu CC, Lin JP, Yang JS, Chou ST, Chen SC, Lin YT, Lin HL. Chung JC. Capsaicin induced cell cycle arrest and apoptosis in human esophagus epithelial carcinoma CE 81T/VGH cells through the elevation of intracellular reactive oxygen species and Ca2+ productions and caspase-3 activation. Mutat Res, 601: 71–82, 2006.
10. Boatright KM, Renatus M, Scott FL, Sperandio S, Shin H, Pedersen IM, Ricci JE, Edris WA, Sutherlin DP, Green DR and Salvesen GS. A unified model for apical caspase activation. Mol Cell, 11: 529–541, 2003.
11. Dierckx K, Zinger R, Kohl A, Lux CJ, Erber R. Gene expression profile of compressed primary human cementoblasts before and after IL-1β stimulation. Clin Oral Investig, 18: 1925–1939, 2014.
12. Nakano Y, Yamaguchi M, Fujita S, Asano M, Saito K, Kasai K. Expressions of RANKL/RANK and M-CSF/c-fms in root resorption lacunae in rat molar by heavy orthodontic force. Eur J Orthod, 33: 335–343, 2011.
13. Hatai T, Yokozeki M, Funato N, Baba Y, Moriyama K, Ichijo H, Kuroda T. Apoptosis of periodontal ligament cells induced by mechanical stress during tooth movement. Oral Dis, 7: 287–290, 2001.
14. Kitagawa M, Tahara H, Kitagawa S, Oka H, Kudo Y, Sato S, Ogawa I, Miyaiichi M, Takata T. Characterization of established cementoblast-like cell lines from human cementum-lining cells in vitro and in vivo. Bone, 39: 1035–1042, 2006.
15. Yamaguchi M, Aihara N, Kojima T, Kasai K. RANKL increase in compressed periodontal ligament cells from root resorption. J Dent Res, 85: 751–756, 2006.
16. Rodriguez-Enfedaque A, Delmas E, Guillaume A, Gaumer S, Mignotte B, Vayssière JL, Renaud F. zVAD-fmk upregulates caspase-9 cleavage and activity in etoposide-induced cell death of mouseembryonic fibroblasts. Biochim Biophys Acta, 1823: 1343–1352, 2012.
17. Matsuzawa H, Toriya N, Nakao Y, Konno-Nagasaka M, Arakawa T, Okayama M, Mizoguchi I. Cementocyte cell death occurs in rat cellular cementum during orthodontic tooth movement. Angle Orthod, 87: 416–422, 2017.
18. Amanda B, Parrish, Christopher D, Freel, and Sally Kornbluth. Cellular mechanisms controlling caspase activation and function. Cold Spring Harb Perspect Biol, 5: a008672, 2013.
19. Fujita S, Yamaguchi M, Utsunomiya T, Yamamoto H, Kasai K. Low-energy laser stimulates tooth movement velocity via expression of RANK and RANKL. Orthod Craniofac Res, 11: 143–155, 2008.
20. Szymczyk KH, Freeman TA, Adams CS, Srinivas V, Steinbeck MJ. Active caspase-3 is required for osteoclast differentiation. J Cell Physiol, 209: 836–844, 2006.
21. Yoshino T, Yamaguchi M, Shimizu M, Yamada K and Kasai K. TNF-zaggravates the progression of orthodontically-induced inflammatory root resorption in the presence of RANKL. J hard Tiss Biol, 23: 155–162, 2014.
22. Kikuta J, Yamaguchi M, Shimizu M, Yoshino T and Kasai K. Notch signaling induces root resorption via RANKL and IL-6 from hPDL cells. J Dent Res, 94: 140–147, 2015.