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Low-energy laser irradiation facilitates the velocity of tooth movement and the expressions of matrix metalloproteinase-9, cathepsin K, and alpha(v) beta(3) integrin in rats

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SUMMARY It has previously been reported that low-energy laser irradiation stimulated the velocity of tooth movement via the receptor activator of nuclear factor kappa B (RANK)/RANK ligand and the macrophage colony-stimulating factor/its receptor (c-Fms) systems. Matrix metalloproteinase (MMP)-9, cathepsin K, and alpha(v) beta(3) [α(v)β3] integrin are essential for osteoclastogenesis; therefore, the present study was designed to examine the effects of low-energy laser irradiation on the expression of MMP-9, cathepsin K, and α(v)β3 integrin during experimental tooth movement.

Fifty male, 6-week-old Wistar strain rats were used in the experiment. A total force of 10*g* was applied to the rat molars to induce tooth movement. A Ga-Al-As diode laser was used to irradiate the area around the moving tooth and, after 7 days, the amount of tooth movement was measured. To determine the amount of tooth movement, plaster models of the maxillae were made using a silicone impression material before (day 0) and after tooth movement (days 1, 2, 3, 4, and 7). The models were scanned using a contact-type three-dimensional (3-D) measurement apparatus. Immunohistochemical staining for MMP-9, cathepsin K, and integrin subunits of α(v)β3 was performed. Intergroup comparisons of the average values were conducted with a Mann–Whitney *U*-test for tooth movement and the number of tartrate-resistant acid phosphatase (TRAP), MMP-9, cathepsin K, and integrin subunits of α(v)β3-positive cells.

In the laser-irradiated group, the amount of tooth movement was significantly greater than that in the non-irradiated group at the end of the experiment ($P < 0.05$). Cells positively stained with TRAP, MMP-9, cathepsin K, and integrin subunits of α(v)β3 were found to be significantly increased in the irradiated group on days 2–7 compared with those in the non-irradiated group ($P < 0.05$).

These findings suggest that low-energy laser irradiation facilitates the velocity of tooth movement and MMP-9, cathepsin K, and integrin subunits of α(v)β3 expression in rats.

Introduction

Recently, various biostimulatory effects of low-energy laser irradiation have been reported, including wound healing (Fung *et al*., 2003; Maiya *et al*., 2005), fibroblast proliferation (Yu *et al*., 2003), collagen synthesis (Reddy *et al*., 1998; Poon *et al*., 2005), and nerve regeneration (Mohammed *et al*., 2007). In particular, the acceleration of bone regeneration by laser treatment has been the focus of recent studies (Merli *et al*., 2005; Pinheiro and Gerbi, 2006).

In orthodontics, low-energy laser irradiation can be utilized for several different treatments, such as the reduction of post-adjustment pain (Lim *et al*., 1995). The stimulatory effects of low-energy laser irradiation on bone regeneration in the median palatal suture area during rapid maxillary expansion in rats have been noted (Saito and Shimizu, 1997). Further, Ozawa *et al*. (1998) demonstrated that laser irradiation stimulates cellular proliferation and differentiation of osteoblast lineage nodule-forming cells, especially in committed precursors, resulting in an increase in the number of differentiated osteoblastic cells as well as in bone formation. Kawasaki and Shimizu (2000) reported that low-energy laser irradiation stimulated the amount of tooth movement and formation of osteoclasts on the pressure side during experimental tooth movement *in vivo*. Fujita *et al*. (2008) and Yamaguchi *et al*. (2007) demonstrated that low-energy laser irradiation stimulated the velocity of tooth movement via the receptor activator of nuclear factor kappa B (RANK)/RANK ligand (RANKL) and the macrophage colony-stimulating factor (M-CSF)/its receptor (c-Fms) expressions.

Osteoclasts are specialized members of the monocyte/macrophage family that differentiate from haematopoietic precursors (Roodman, 1996). The expression of tartrate-resistant acid phosphatase (TRAP) is a characteristic of the macrophage/osteoclast lineage and is often used as a lineage marker (Roodman *et al*., 1985). Activity of osteoclasts *in vitro* is measured by excavation of pits in bone or dentine slices, and this is the feature that undoubtedly identifies mature active osteoclasts (Chambers *et al*., 1984). Among matrix metalloproteinases (MMPs), MMP-9 is known as

one of the major proteases produced by osteoclasts (Okada *et al*., 1995). MMP-9 was shown to be the main MMP involved in the invasive activity of osteoclasts (Delaissé *et al*., 2000). Among cysteine proteinases, cathepsin K plays an essential role in osteoclast-mediated degradation of bone organic matrix (Tezuka *et al*., 1994). Knockout of the enzyme in mice, as well as a lack of functional enzyme in the human pathological condition, pycnodysostosis, results in osteopetrosis (Gelb *et al*., 1996). Ohba *et al*. (2000) reported that cathepsin K expression was detected in mono- and multinuclear osteoclasts on the pressure side of the alveolar bone 12 hours after force application during experimental tooth movement in rats.

Integrins are heterodimeric adhesion receptors that mediate cell–matrix interaction. Hultenby *et al*. (1993) indicated that the alpha(v) beta(3) [α(v)β3] integrin mediates tight attachment of the osteoclast to the bone matrix. Osteoclasts exhibit high expression of the α(v)β3 integrin, which binds to a variety of extracellular matrix proteins, including vitronectin, osteopontin, and bone sialoprotein (Nakamura *et al*., 2007). Talic *et al*. (2004) reported that the α(v)β3 integrin adhesion receptor is expressed during experimental tooth movement. Therefore, MMP-9, cathepsin K, and α(v)β3 integrin may be involved in bone turnover during orthodontic tooth movement.

However, little is known about the effects of laser irradiation on osteoclastogenesis via MMP-9, cathepsin K, and α(v)β3 integrin. The present study was designed to examine the effects of low-energy laser irradiation on MMP-9, cathepsin K, and α(v)β3 integrin expressions during experimental tooth movement.

Materials and methods

Experimental animals and tooth movement

The animal experimental protocol in this study was approved by the Ethics Committee for Animal Experiments at the Nihon University School of Dentistry at Matsudo (approval no. ECA-05-0025).

A total of 50 male, 6-week-old Wistar strain rats (Sankyo Labo Service Co., Tokyo, Japan) weighing 180 ± 10 g were used for the experiment. They were kept in the animal centre of the Nihon University School of Dentistry at Matsudo in separate cages in a 12 hour light/dark environment at a constant temperature of 23°C and provided with food and water *ad libitum*. The health status of each rat was evaluated by daily body weight monitoring for 1 week before the start of the experiment.

Experimental tooth movement was performed according to the method of Kawasaki and Shimizu (2000), with a closed-coil spring (wire size: 0.005 inch, diameter: 1/12 inch; Accurate Sales Co., Chiba, Japan) ligated to the maxillary first molar cleat by a 0.008 inch stainless steel ligature wire (Tomy International Inc., Tokyo, Japan). The other side of the coil spring was also ligated, with the holes in the maxillary incisors drilled laterally just above the gingival papilla with a No.1/4 round bur, using the same ligature wire. The upper first molar was moved mesially by the closed-coil spring with a force of 10 g. The 50 rats were divided into two equal groups to form a laser-irradiated and non-irradiated group. All surgery was carried out under general anaesthesia using an intraperitoneal injection of mixed ketamine hydrochloride and xylazine hydrochloride. The period of the experiment was 7 days.

Laser irradiation

A Ga-Al-As diode laser (Osada Inc., Tokyo, Japan) with a wavelength of 810 nm and continuous waves at 100 mW output power was used in the study. These irradiation conditions were determined by previous experiments (Kawasaki and Shimizu, 2000). The laser beam was delivered by a 0.6 mm diameter optical fibre, and irradiation was administered, under anaesthesia, by placing the end of the optical fibre tip in contact with the mesial, buccal, and palatal sides of the gingiva, located in the area of the upper right first molar undergoing orthodontic tooth movement (Figure 1). Irradiation was performed for 3 minutes at each point (a total of 9 minutes) once a day on days 0–7 (a total of eight times). The total energy corresponding to an exposure time of 9 minutes was 54.0 J, which is similar to the dose used in a previous study (Yamaguchi *et al*., 2007).

Measurement of tooth movement

To measure the amount of tooth movement, plaster models of the maxillae were made using a silicone impression material (Dent Silicone-V; Shofu Inc., Kyoto, Japan) before (day 0) and after tooth movement (days 1, 2, 3, 4, and 7).

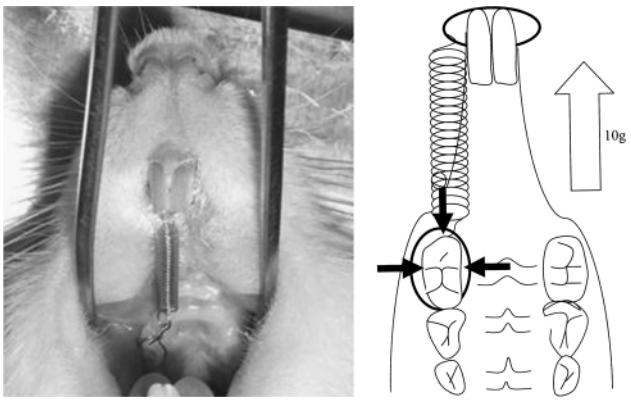

Figure 1 Experimental tooth movement was performed with a closed-coil spring (wire size: 0.005 inch, diameter: 1/12 inch) ligated to the maxillary first molar cleat by a 0.008 inch stainless steel ligature wire. The other side of the coil spring was also ligated, with the holes in the maxillary incisors drilled laterally just above the gingival papilla with a no.1/4 round bur, using the same ligature wire. The upper first molar was moved mesially by the closed-coil spring with a force of 10 g. The period of the experiment was 7 days.

The models were scanned using a contact-type three-dimensional (3D) measurement apparatus (3D-picza; Roland Co., Hamamatsu, Japan) by setting the plane to pass through three points, which were the bilateral interpapillary crests between the first and second molars and the interpapillary crest between the second and third molars.

Using 3D morphological analysis software (3D-Rugle; Medic Engineering Inc., Kyoto, Japan), the distance between the central fossa of the first molar and the mesial surface of the second molar was measured to determine tooth movement (Figure 2).

Tissue preparation

The experimental periods were set at 1, 2, 3, 4, and 7 days after tooth movement. The rats were divided into 10 equal groups: a laser-irradiated and a non-irradiated group (days 0–1, 0–2, 0–3, 0–4, and 0–7).

All rats were killed by an overdose of mixed ketamine hydrochloride and xylazine hydrochloride and perfused with 10 per cent formalin in 0.1 M phosphate buffer transcardially, after which the maxilla was immediately dissected and immersed in the same fixative overnight at 4°C. The specimens were decalcified in 10 per cent disodium ethylenediamine tetra-acetic acid (pH 7.4) solution for 4 weeks; the decalcified specimens were then dehydrated through an ethanol series and embedded in paraffin. Each sample was sliced into 4 μm continuous sections in the horizontal direction and prepared for haematoxylin and eosin (H&E), TRAP, and immunohistochemistry staining for MMP-9, cathepsin K, and integrin subunits of α(v)β3.

The periodontal tissues in the pressure areas, which were one-quarter of the mesial area, were facing the mesial root, as determined when linked with the centre of the mesial root and the distobuccal root of the first molar (Figure 3). To evaluate the effect of laser irradiation on the formation of multinucleated osteoclasts, the number of multinucleated osteoclasts in the irradiated and non-irradiated groups was counted.

Immunohistochemistry of MMP-9, cathepsin K, and integrin subunits of α(v)β3

Immunohistochemical staining was performed as follows: the sections were deparaffinized and endogenous peroxidase activity was quenched by incubation in 3 per cent H_2O_2 in methanol for 15 minutes at room temperature. After washing in Tris-buffered saline (TBS), the sections were incubated with the polyclonal anti-rabbit MMP-9 (Abcam, Cambridge, UK; working dilution: 1:300), the polyclonal anti-goat cathepsin K (Santa Cruz Biotechnology Inc., Santa Cruz, California, USA; working dilution: 1:300), the polyclonal anti-rabbit α(v) integrin subunit (Chemicon International Inc., Temecula, California, USA; working dilution: 1:1500), and β3 integrin subunit (Chemicon International Inc.; working dilution: 1:150) overnight at 4°C. MMP-9, cathepsin K, and integrin subunits of α(v) and β3 were stained using Histofine Simple Stain Rat MAX-Po(R) kits (Nichirei, Tokyo, Japan) according to the manufacturer's protocol. The

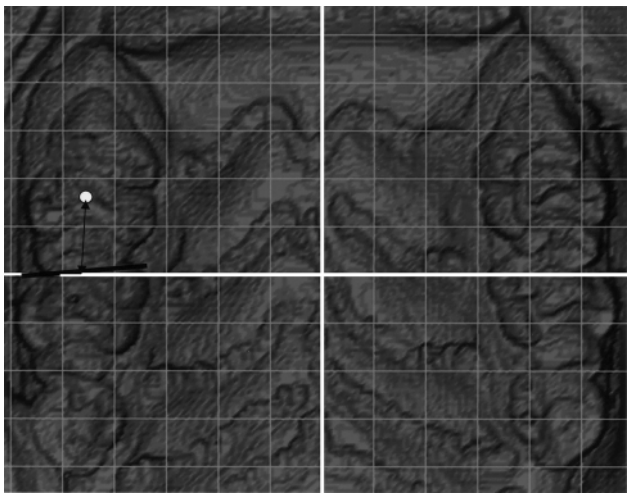

Figure 2 Measurement of tooth movement. The plaster models were scanned using a contact-type three-dimensional (3D) measurement apparatus at the bilateral interpapillary crest between the first and second molars and the interpapillary crest between the second and third molars. Using 3D morphological analysis software, the distance between the first molar central fossa and the second molar mesial surface was measured.

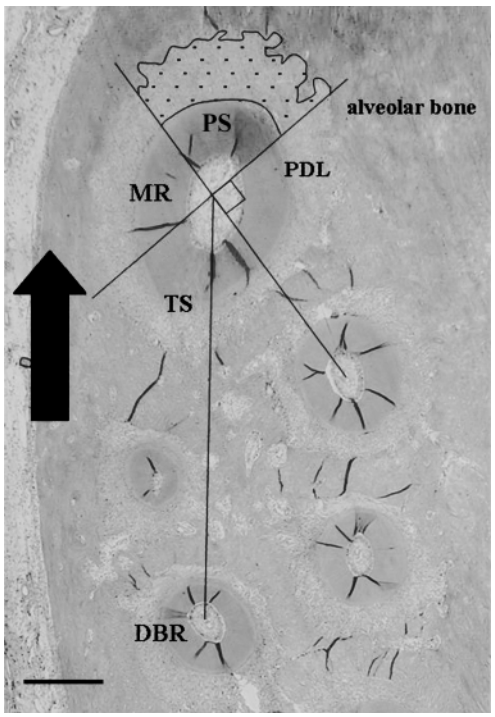

Figure 3 Photograph of labelled alveolar bone taken under a fluorescent microscope. The number of multinucleated osteoclasts, tartrate-resistant acid phosphatase, matrix metalloproteinase-9, cathepsin K, and alpha(v) and beta(3) integrin-positive osteoclasts was measured on the pressure side (PS) at a distance of one-quarter in height of the mesial area of the root (dots) opposite to the tension side (TS). Arrow, tooth movement direction; MR, mesial root; DBR, distal buccal root; PDL, periodontal ligament. Bar = 50 μm.

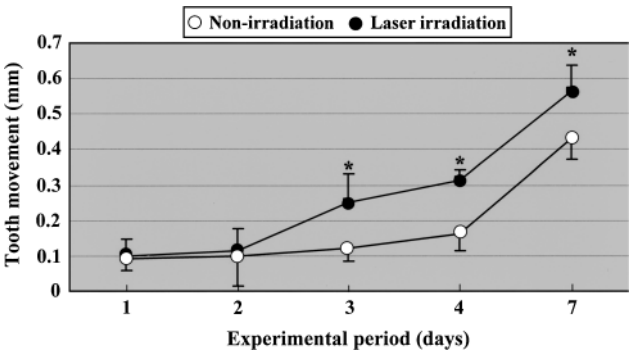

Figure 4 Effect of laser irradiation on tooth movement. Values are mean ± standard deviation of five rats. The amount of tooth movement was significantly greater in the irradiated group on days 3, 4, and 7 compared with that in the non-irradiated group. *P < 0.05.

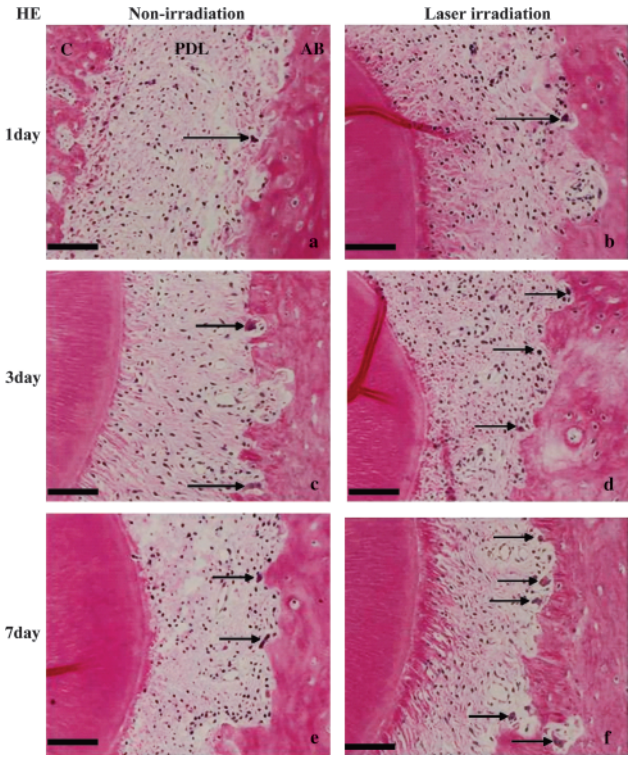

Figure 5 Light microscopy images of the effects of laser irradiation on multinucleated osteoclasts after tooth movement [haematoxylin and eosin, original magnification ×400]. Day 1: resorption lacunae with a few multinucleated osteoclasts (arrows) were observed on the surface of the alveolar bone and root in the non-irradiated and irradiated groups (a and b). Day 3: many resorption lacunae with multinucleated osteoclasts appeared on the alveolar bone surface. The number of multinucleated osteoclasts in the irradiated group was more than that in the non-irradiated group (c and d). Day 7: on the surface of the alveolar bone, bone resorption lacunae with multinucleated osteoclasts were increased in comparison with those on day 3 in both groups (e and f). AB, alveolar bone; PDL, periodontal ligament; C, cementum. Bar = 50 μm.

sections were rinsed with TBS and final colour reactions were performed using the substrate reagent 3,3′-diaminobenzidine tetra-hydrochloride and aminoethyl carbazole. They were then counterstained with H&E. As

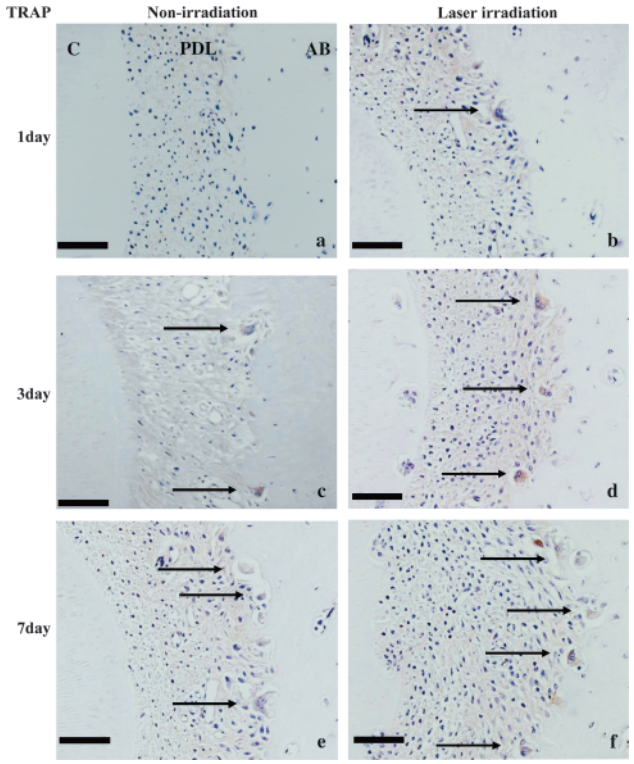

Figure 6 Effects of laser irradiation on tartrate-resistant acid phosphatase (TRAP)-positive osteoclasts after tooth movement as shown by immunohistochemistry (original magnification ×400). Day 1: resorption lacunae with a few TRAP-positive multinucleated osteoclasts were observed on the surfaces of the alveolar bone and root (a and b). TRAP immunoreactivity (arrows) was observed in osteoclasts on the alveolar bone surface in the laser-irradiated group (d and f) on days 3 and 7, as well as on days 3 and 7 in the non-irradiated group (c and e). AB, alveolar bone; PDL, periodontal ligament; C, cementum. Bar = 50 μm.

immunohistochemical controls, some sections were incubated in the same way and then further incubated with either non-immune rabbit immunoglobulin G or 0.01 M phosphate-buffered saline alone instead of the primary antibody.

To evaluate the effect of laser irradiation on the expressions of TRAP, MMP-9, cathepsin K, and integrin subunits of α(v) and β3, the number of positive cells in the irradiated and non-irradiated groups was counted.

Statistical analysis

Intergroup comparisons of the average values were conducted for tooth movement and the number of TRAP, MMP-9, cathepsin K, and integrin subunits of α(v)β3-positive cells with a Mann–Whitney *U*-test. A *P* value of less than 0.05 was considered significant.

Results

Tooth movement during the experimental period

The body weight of the rats in both groups decreased transiently on day 1 and then recovered. No significant

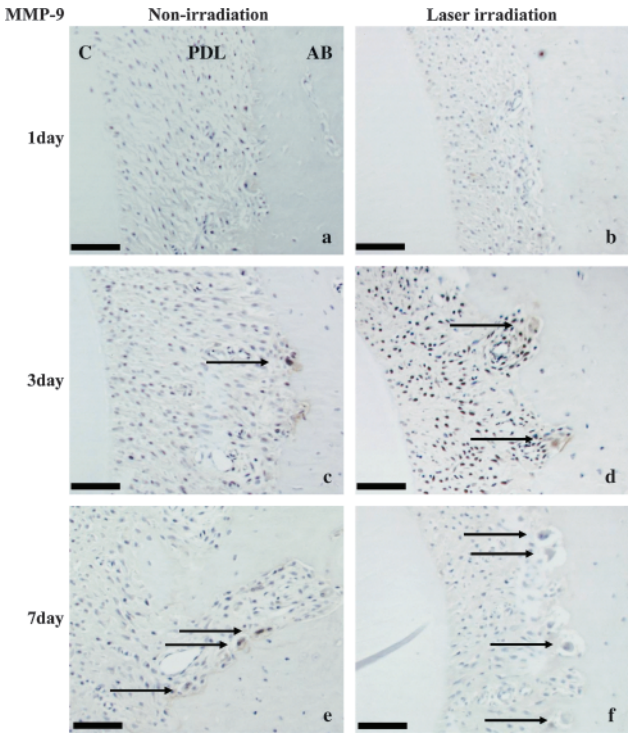

Figure 7 Effect of laser irradiation on matrix metalloproteinase (MMP)-9-positive osteoclasts by immunohistochemistry (original magnification ×400). On day 1 after tooth movement, no MMP-9-positive osteoclasts at the bone surface were observed in either the non-irradiated or the irradiated group (a and b). Immunoreactivity to MMP-9 was observed in the osteoclasts on the alveolar bone surface in the irradiated group (d and f) on days 3 and 7 and on days 3 and 7 in the non-irradiated group (c and e). AB, alveolar bone; PDL, periodontal ligament; C, cementum. Bar = 50 μm.

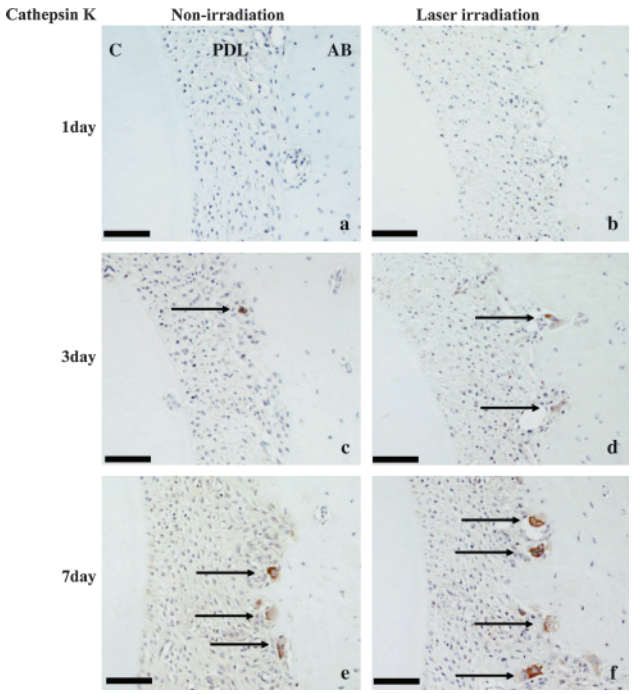

Figure 8 Effect of laser irradiation on cathepsin K-positive osteoclasts by immunohistochemistry (original magnification ×400). On day 1 after tooth movement, no cathepsin K-positive osteoclasts were observed at the bone surface in the non-irradiated or irradiated groups (a and b). Immunoreactivity of cathepsin K (arrows) was observed in the osteoclasts on the alveolar bone surface in both the irradiated group (d and f) and the non-irradiated group (c and e) on days 3 and 7. AB, alveolar bone; PDL, periodontal ligament; C, cementum. Bar = 50 μm.

differences between the two groups were observed (data not shown). The amount of tooth movement was significantly greater in the irradiated group on day 3 (2.0-fold), day 4 (1.9-fold), and day 7 (1.3-fold) compared with that in the non-irradiated group (Figure 4).

Histological changes in periodontal tissues during tooth movement

Inflammation in periodontal tissues did not vary significantly in the control and irradiated groups during the experimental period (data not shown). On day 1 of tooth movement, the arrangement of the fibres and fibroblasts had become coarse and irregular, and the blood capillaries were compressed. Resorption lacunae with a few multinucleated osteoclasts were observed on the surface of the alveolar bone and root, both in the irradiated and non-irradiated groups (Figure 6a,b). On day 3, the periodontal ligament (PDL) was composed of a coarse arrangement of fibres and expanded blood capillaries. Many resorption lacunae with multinucleated osteoclasts appeared on the alveolar bone surface, while in the fibres of the PDL, many mononuclear cells were present. The number of multinucleated osteoclasts in the irradiated group was more than that in the non-irradiated group (Figure 5c,d). On day 7, the number of bone resorption lacunae with multinucleated osteoclasts was increased on the surface of the alveolar bone in comparison with those on day 3 in both groups. However, the number of multinucleated osteoclasts in the irradiated group was more than that in the non-irradiated group (Figure 5e,f).

Immunohistochemical findings of TRAP staining

On day 1 of tooth movement, resorption lacunae with a few TRAP-positive multinucleated osteoclasts were observed on the surfaces of the alveolar bone and root (Figure 6a,b). On day 3, many resorption lacunae with TRAP-positive multinucleated osteoclasts appeared on the alveolar bone surface on the pressure side. The number of TRAP-positive cells in the irradiated group was greater than that in the non-irradiated group (Figure 6c,d). On day 7 after tooth movement, bone resorption lacunae with multinucleated TRAP-positive osteoclasts were observed on the surface of the alveolar bone (Figure 6e,f).

Immunohistochemistry of MMP-9, cathepsin K, and integrin subunits of α(v)β3

On day 1 of tooth movement, no MMP-9 or cathepsin K-positive osteoclasts were observed at the bone surface in

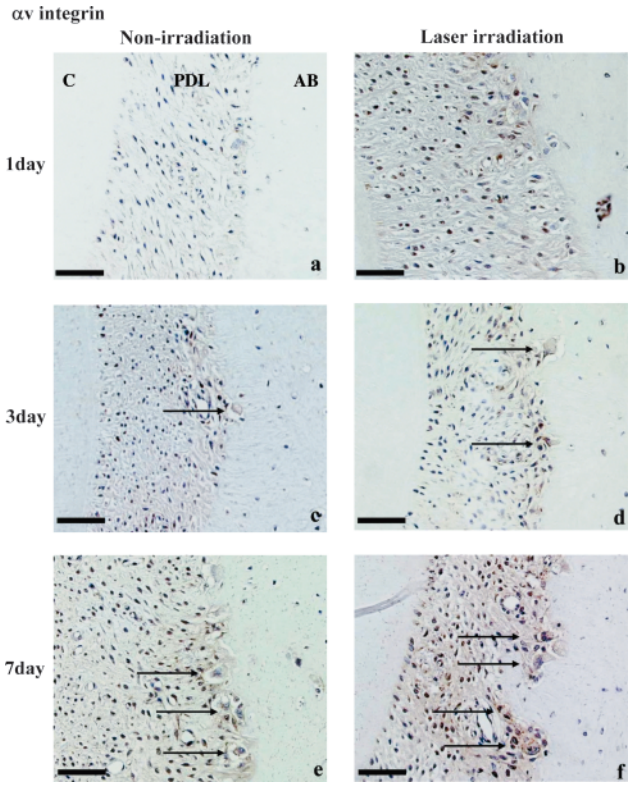

Figure 9 Effect of laser irradiation on alpha(v) [α(v)]-positive osteoclasts after tooth movement by immunohistochemistry (original magnification ×400). Day 1: a few subunits of α(v)-positive osteoclasts were observed in the non-irradiated group (a and b). Immunoreactivity of α(v) (arrows) was observed in the osteoclasts on the alveolar bone surface in the irradiated group (d and f) on days 3 and 7 and in the non-irradiated group (c and e) on days 3 and 7. AB, alveolar bone; PDL, periodontal ligament; C, cementum. Bar = 50 μm.

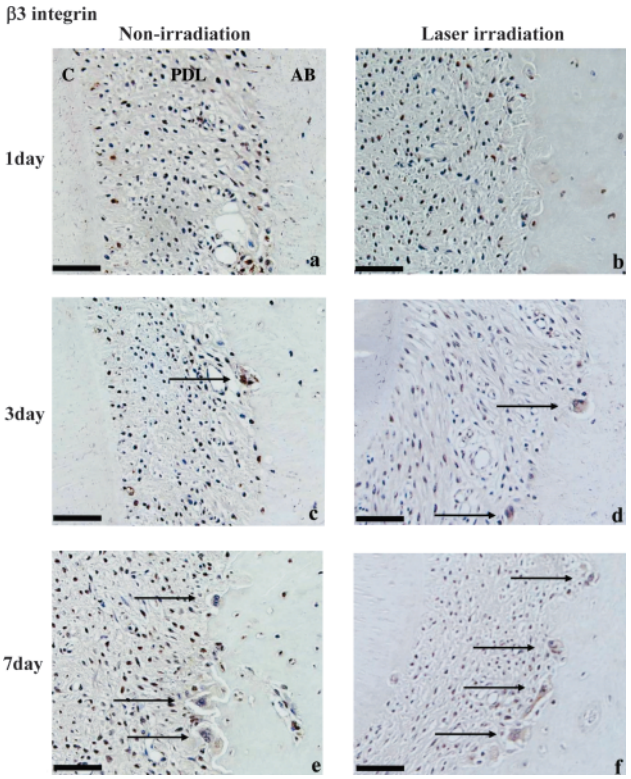

Figure 10 Effect of laser irradiation on beta(3) (β3)-positive osteoclasts by immunohistochemistry (original magnification ×400). On day 1 after tooth movement, a few subunits of β3-positive osteoclasts were observed in the non-irradiated group (a and b). Immunoreactivity of β3 (arrows) was observed in the osteoclasts on the alveolar bone surface in the irradiated group (d and f) and the non-irradiated group (c and e) on days 3 and 7. AB, alveolar bone; PDL, periodontal ligament; C, cementum. Bar = 50 μm.

either the irradiated or the non-irradiated group (Figures 7a,b and 8a,b), but a few subunits of α(v)β3-positive osteoclasts were found in the non-irradiated group (Figures 9a and 10a). On day 2, a few MMP-9 and cathepsin K-positive osteoclasts were observed in the irradiated group. On day 3, the immunoreactivity to MMP-9, cathepsin K, and subunits of α(v)β3 of the osteoclasts increased in the irradiated group. MMP-9, cathepsin K, and subunits of α(v)β3-positive osteoclasts were also observed in the non-irradiated group (Figures 8c; 9c,e; 10c,e; and 11). A large number of MMP-9, cathepsin K, and subunits of α(v)β3-positive osteoclasts were observed on day 4 in both groups and the immunoreactivity to MMP-9, cathepsin K, and subunits of α(v)β3 of the osteoclasts increased. On day 7, immunoreactivity to MMP-9, cathepsin K, and integrin subunits of α(v)β3 was observed in the osteoclasts on the alveolar bone surface in both groups (Figures 7e,f; 8e,f; 9e,f; and 10e,f).

Quantitative evaluation of multinucleated osteoclasts, TRAP, MMP-9, cathepsin K, and integrin subunits of α(v)β3

Quantitative evaluation showed that the number of multinucleated osteoclasts and TRAP-positive cells was significantly increased in the irradiated group on day 2 (multinucleated osteoclasts: 2.1-fold and TRAP: 2.0-fold), day 3 (multinucleated osteoclasts: 1.6-fold and TRAP: 2.0-fold), and day 7 (multinucleated osteoclasts: 1.6-fold and TRAP: 1.2-fold) compared with that in the non-irradiated group (Figure 11a, b).

The number of MMP-9, cathepsin K, and α(v)β3 integrin-positive cells was found to be significantly increased in the irradiated group on day 2 [MMP-9: 4.0-fold, cathepsin K: 2.7-fold, α(v) integrin: 1.9-fold, and β3 integrin: 2.0-fold], day 3 [MMP-9: 2.3-fold, cathepsin K: 1.8-fold, α(v) integrin: 1.8-fold, and β3 integrin: 2.0-fold], and day 7 [MMP-9: 1.8-fold, cathepsin K: 1.6-fold, α(v) integrin: 1.4-fold, and β3 integrin: 1.4-fold] compared with that in the non-irradiated group (Figure 11).

Discussion

In the present study, the amount of tooth movement was significantly greater in the irradiated group on day 3 (2.0-fold) and day 4 (1.9-fold) compared with that in the non-irradiated group (Figure 4). Furthermore, the number

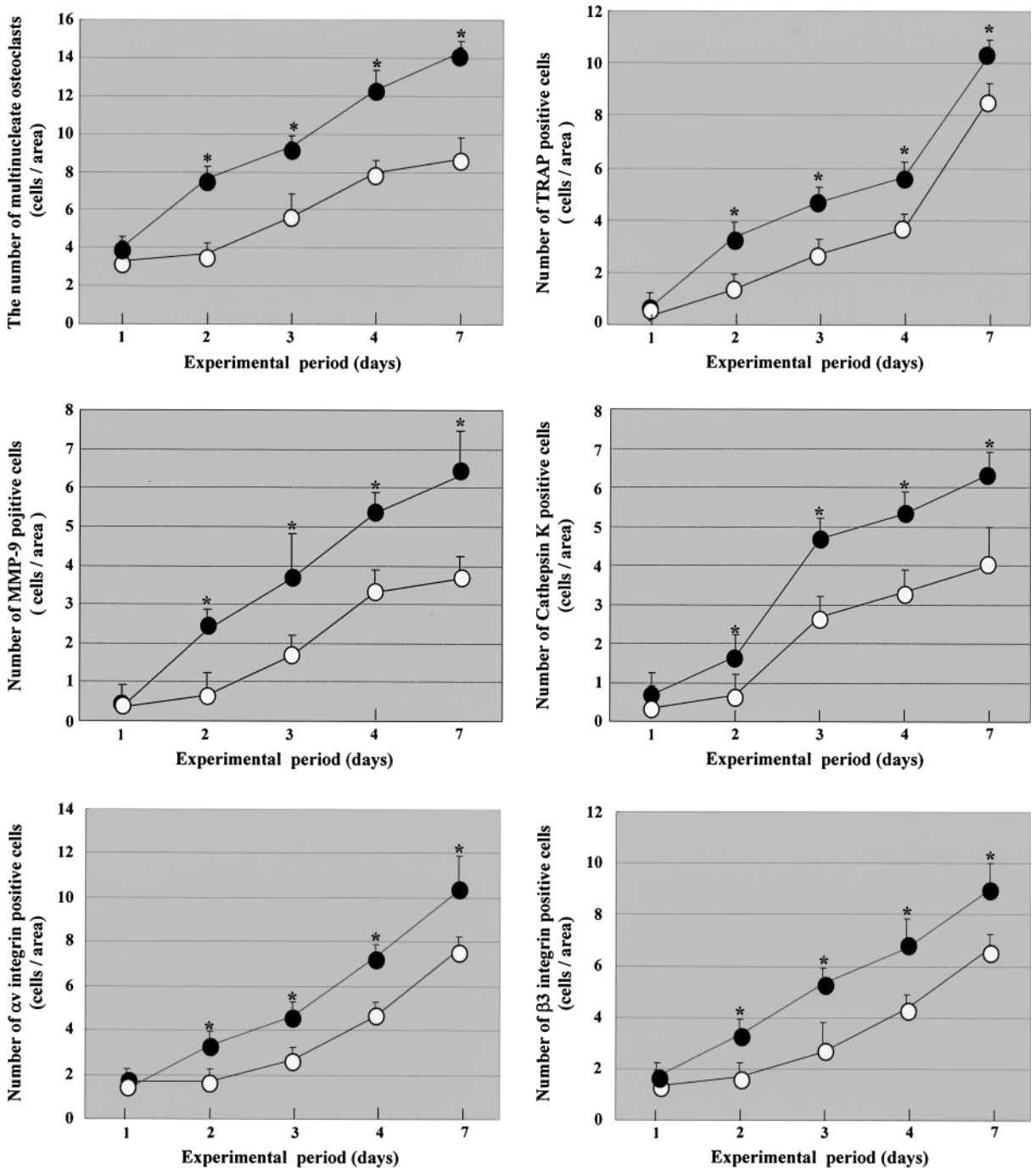

Figure 11 Quantitative evaluation. The number of multinucleated osteoclasts, tartrate-resistant acid phosphatase, matrix metalloproteinase-9, cathepsin K, and integrin subunits of alpha(v) and beta(3) in the irradiated group increased compared with that in the non-irradiated group on days 2, 3, 4, and 7. Values are mean ± standard deviation of five rats. *$P < 0.05$. Closed circles, irradiated group; open circles, non-irradiated group.

of multinucleated osteoclasts and TRAP-positive cells in the irradiated group was more than that in the non-irradiated group (Figures 6 and 7). Recent studies have demonstrated that low-energy irradiation accelerates orthodontic tooth movement in rats (Kawasaki and Shimizu, 2000) and humans (Cruz *et al*., 2004). Kawasaki and Shimizu (2000) showed that orthodontic movement of laser-irradiated rat teeth was 30 per cent faster compared with that in non-irradiated rats, due to an increase in bone formation at the tension side and in the number of osteoclasts at the compression side as a result of cellular stimulation promoted by low-energy laser irradiation.

The effects of low-energy laser irradiation on MMP-9, cathepsin K, and α(v)β3 integrin expressions *in vivo* in the present study (Figures 7-11) show that laser irradiation increased the expression of these positive cells at the compression side on days 2–7 (1.4- to 4.0-fold). This histological phenomenon, similar to that found by Fujita *et al*. (2008) and Yamaguchi *et al*. (2007), demonstrates that low-energy laser irradiation stimulates the expressions of RANK/RANKL (1.3- to 1.5-fold) and M-CSF/c-Fms (1.2- to 1.7-fold) compared with that in the non-irradiated group at the compression side during orthodontic tooth movement. These findings are in agreement with the results of those studies.

It has recently been demonstrated that MMP-9 and cathepsin K expressions are induced in osteoclasts by RANKL (Oshiro *et al*., 2001; Wittrant *et al*., 2003). Furthermore, c-Fms and α(v)β3 integrins collaborate during osteoclast differentiation (Faccio *et al*., 2003). Previous studies have demonstrated that MMP-9, cathepsin K, and α(v)β3 integrin involve osteoclast differentiation (Hultenby *et al*., 1993; Faccio *et al*., 2003; Nakamura *et al*., 2007), and these factors are expressed on osteoclasts during orthodontic tooth movement (Ohba *et al*., 2000; Talic *et al*., 2004). Furthermore, Aihara *et al*. (2006) and Fujita *et al*. (2008) reported that low-energy laser irradiation facilitates differentiation and activation of osteoclasts via RANK and c-Fms gene expressions *in vitro*. Therefore, the acceleration of orthodontic tooth movement by low-energy laser irradiation may be affected not only by RANK/RANKL and M-CSF/c-Fms systems but also by MMP-9, cathepsin K, and α(v)β3 integrin. Further studies are necessary to confirm the effects of laser irradiation on gene expressions of MMP-9, cathepsin K, and α(v)β3 integrin in osteoclasts *in vitro*.

Regarding the tension side during orthodontic tooth movement, Ozawa *et al*. (1998) reported that laser irradiation in the early stages of osteoblast-like cells isolated from foetal rat calvariae significantly stimulated cellular proliferation, alkaline phosphatase activity, and osteocalcin gene expression. They also found that laser irradiation in the early stages of culture significantly stimulated the proliferation of osteoblasts, resulting in the development of a greater number (1.7-fold) and larger area (3.4-fold) of bone nodules in the culture dish on day 21. These results suggest that laser irradiation may stimulate proliferation and differentiation, resulting in an increase in the number of differentiated osteoblastic cells and an increase in bone formation. Furthermore, Barushka *et al*. (1995) reported that low-energy laser (He–Ne) irradiation after injury affected the population of osteoblasts and osteoclasts at the injured site. On the basis of the findings of the present study, it is possible that low-energy laser irradiation may accelerate the process of bone remodelling by stimulating osteoblast and osteoclast differentiation during orthodontic tooth movement.

Conclusions

Low-energy laser irradiation facilitates the velocity of tooth movement and MMP-9, cathepsin K, and integrin subunits of α(v)β3 expressions in rats.

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