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Effects of alendronate on restoration of biomechanical properties of periodontium in replanted rat molars

T. Shibata[1], K. Komatsu[1], A. Shimada[1], S. Shimoda[2], S. Oida[3], K. Kawasaki[2], M. Chiba[1]
Departments of [1]Pharmacology, [2]Anatomy I and [3]Biochemistry, School of Dental Medicine, Tsurumi University, Tsurumi-ku, Yokohama, Japan

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Objective: We examined the effect of the pretreatment of roots with alendronate on the restoration of the support function of the healing periodontal ligament in replanted rat molars.

Methods: The left maxillary first molars were extracted, placed in 0.9% NaCl containing 1 mM alendronate (alendronate group) or 0.9% NaCl (control group) for 5 min, and were replanted into their sockets. Groups of animals were killed at 7, 14, and 21 days after replantation. Normal control rats were also killed on the same days. The force required to extract the replanted or normal tooth from its socket was measured, and a load–deformation curve was developed and analyzed. Micro-computed tomography and histologic analyses were also made.

Results: The mechanical properties of the healing periodontal ligament in the alendronate group were gradually restored from 7 to 21 days. However, fractures of the roots and bones during mechanical testing occurred in most of the replanted teeth in the control group at 21 days. The rates of restoration of the mechanical strength, extensibility, stiffness, and toughness for the alendronate group at 21 days were 67, 98, 74, and 68% of the normal controls, respectively. Micro-computed tomography and histologic observations revealed that bone-like structures within the pulp and ankylosis between the roots and socket bones occurred commonly in the control group, but were uncommon in the alendronate group.

Conclusions: Our findings suggest that the pretreatment with alendronate inhibits the formation of abnormal mineralized tissues and results in better restoration of the support function of the healing periodontal ligament in replanted teeth.

T. Shibata, Department of Pharmacology, School of Dental Medicine, Tsurumi University, 2-1-3 Tsurumi, Tsurumi-ku, Yokohama 230-8501, Japan
Tel: +81 45 580 8453
Fax: +81 45 573 9599
e-mail: shibata-t@tsurumi-u.ac.jp

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Tooth replantation and transplantation are becoming less common procedures with the advent of dental implants. On the other hand, it has been pointed out that replantation and autotransplantation are reasonable treatment options in properly selected cases such as traumatic injuries to newly erupted permanent anterior teeth and natural replacements for failing or missing teeth (1, 2). Replanted or autotransplanted teeth will have a periodontal ligament attachment, which serves as a shock absorber, provides proprioception and adaptation for tooth movement, and can promote bone formation (1, 2). These may be advantages over dental implants.

The main problems in tooth replantation appear to be resorption and ankylosis of the alveolar bones and teeth, according to various animal models (3–5) and human studies (1, 2). It has been reported that pretreatment of root surfaces with stannous fluoride and tetracycline resulted in complete absence of inflammatory resorption and ankylosis after 21 days in replanted dog teeth (6).

Levin *et al*. (7) reported that topical treatment of root surfaces with alendronate, a bisphosphonate, resulted in better healing than treatment with saline in replanted dog teeth, with the alendronate treatment producing less loss of root mass due to resorption after 4 months. This suggests that bisphosphonates may reduce resorption and ankylosis, leading to better restoration of the periodontal ligament between the roots and socket bones in replanted teeth.

From a functional point of view, the biomechanical properties of a wound are among the most important properties in the wound healing process (8). The functional restoration of the periodontal ligament has been investigated by measuring the mechanical properties of the healing periodontal ligament after extrusive luxation of the tooth (9), orthodontic retention (10), and tooth replantation (11). Measurement of the mechanical properties may provide useful information for a quantitative evaluation of the restoration of the tooth supporting function of the periodontal ligament (11).

The aims of the present study were to examine changes in the mechanical properties of the periodontal ligament after replantation of rat molars pretreated with alendronate, in relation to morphology, and to evaluate the effect of alendronate on the restoration of the mechanical properties of the periodontal ligament in replanted teeth.

Material and methods

Replantation of rat maxillary molar

One hundred male Wistar rats, aged 34–36 days, with an average body weight of 113 ± 7 g, were used. They were fed a powdered diet (CE-2, Nihon Clea, Tokyo, Japan) and given water *ad libitum* during the experimental period. At 41–43 days of age, they were initially divided into normal control (40 rats), control (30 rats), and alendronate (30 rats) groups. The animals of the control and alendronate groups were anesthetized with intraperitoneal injections of ketamin hydrochloride (47 mg/kg body weight; Fuji Chemical, Saitama, Japan) and medetomidine hydrochloride (0.4 mg/kg body weight; Orion, Espoo, Finland). Then, their left maxillary first molars were extracted using extraction forceps, and were placed in saline (0.9% NaCl solution) containing 1 mM alendronate (Teiroc®Inj., TEIJIN, Osaka, Japan; alendronate group) or saline (control group) for 5 min (7) at room temperature of approximately 20°C. The molars were then put back (replanted) into their sockets. There was approximately 10 min between the extraction and replantation procedures. Then, the rats were subcutaneously injected with benzylpenicillin procaine [300,000 U/ml, 0.67 ml/100 g body weight (4); Tamura Pharmaceuticals, Tokyo, Japan], and were awakened with an intraperitoneal injection of atipamezole hydrochloride (0.83 mg/kg body weight; Orion). When rats died and/or the tooth crown or root was broken during the replantation procedures, the data from these rats were excluded. In the normal control group of rats, no extraction or replantation of their maxillary first molars was performed.

Biomechanical analysis

Mechanical testing — Groups of nine to 10 animals were killed with an overdose of ether at 7, 14, and 21 days after replantation. In the normal control group, 10 rats were killed at 0, 7, 14, and 21 days. In each rat, immediately after death, its maxilla was dissected out, the adherent soft tissues were removed, and the maxilla was kept in saline at 4°C. The left maxillary first molar was extracted from its socket according to the method described by Chiba and Ohkawa (12) using a materials testing machine (Autograph type S-100, Shimadzu Seisakusho, Kyoto, Japan) at a speed of 5 mm/min. A load–deformation curve of the periodontal ligament of the tooth was then obtained and stored in a computer (VAIO, Sony, Tokyo, Japan) with the aid of an amplifier (PA-011, Star Medical, Tokyo, Japan) and an analog-to-digital converter (Model ESC-1312, Star Medical). All experiments were performed at room temperature (22–26°C). The time between killing the animals and mechanical testing ranged from 24 to 96 min. When the tooth crown, the root, or the socket bone was broken during mechanical testing, the data from such samples were excluded.

Analysis of load–deformation curves — From each load–deformation curve, the following biomechanical measures were estimated (13, 14): (i) maximum shear load (the peak value; mechanical strength); (ii) maximum shear deformation (the distance pulled to reach the maximum shear load; extensibility); (iii) tangent modulus (the slope of the linear region of the curve; stiffness); (iv) failure energy in shear (the area between the load–deformation curve and x-axis to the peak value; toughness).

Radiographic analysis — Radiographs of extracted teeth and jaws were taken in a soft X-ray apparatus (Type EMB, Softex, Tokyo, Japan). Fractures of alveolar bones and/or teeth during mechanical testing were examined by analyzing those radiographic images and by stereomicroscopic observations of extracted teeth and alveolar bones. We also measured the distance from the cemento-enamel junction to the root tip along the long axis of the mesial root of the extracted tooth on the radiographic images (15).

Morphological analysis

For morphological analysis, another 22 male Wistar rats, 34–36 days, were used. They were divided into normal control (eight rats), control (seven rats), and alendronate (seven rats) groups. Extraction of the left maxillary molar and replantation into its original socket were done with the same procedures as in the control and alendronate groups for the biomechanical analysis, described above. Under anesthesia with ketamine and medetomidine, the control and alendronate groups were perfused with 0.1 M phosphate buffer (pH 7.2) containing 4% paraformaldehyde at 7, 14, and 21 days after replantation. The normal control group was also perfused at 0, 7, 14, and 21 days. The maxillae were dissected and then placed in the same fixative as above at 4°C.

Micro-computed tomography — Each maxilla containing a replanted left first molar was horizontally scanned at 16-μm intervals with a micro-computed tomography system (MCT-CB100MFZ, Hitachi Medico, Tokyo, Japan), and 201 images (480 × 480 pixels) were obtained. Two- and three-dimensional analyses were made to determine the levels of ankylosis and resorption of the bones and roots in the replanted and normal control teeth.

Histology — After the micro-computed tomography analysis, the maxillae were decalcified in 14% EDTA for 3 weeks, dehydrated, and embedded in paraffin. Sagittal sections were cut serially with a microtome (Model 1320, Leica Instruments, Germany) setting of 6 μm, were stained with hematoxylin and eosin, and were observed by ordinary and polarized light microscopes.

Statistics

The chi-squared test was used to examine differences in the numbers of fractures during mechanical testing between the two groups. The changes in the length of the mesial root, and biomechanical measures at 7, 14, and 21 days after replantation were examined by a one-way analysis of variance (ANOVA). The differences in the length of the mesial root, and biomechanical measures in the normal control, control, and alendronate groups were examined by a *t*-test and, when appropriate, by Scheffé's method.

Table 1. Numbers of fractures of roots and/or alveolar bones during mechanical testing

Days after replantation	Normal control group	Control group	Alendronate group
0	1 (10)	–	–
7	0 (10)	0 (9)	0 (10)
14	1 (10)	3 (9)	0 (10)
21	0 (10)	8 (9)[a,b]	2 (9)

Number of rats is shown in parentheses. Significant differences [a]from alendronate group, $p < 0.005$ and [b]from normal control group, $p < 0.001$ (chi-squared test).

Results

Fractures of bone and root during mechanical testing

Table 1 shows the number of fractures of roots and/or alveolar bones that occurred during mechanical testing. In the normal control group, we were able to extract the maxillary first molars without fractures in all but two teeth that had fractures of the mesial roots at 0 and 14 days. In the control group, no fractures were observed at 7 days, but were observed at 14 days (33%) and 21 days (89%). The number of fractures at 21 days in the control group was significantly greater than that in either the alendronate group ($p < 0.005$, chi-squared test) or the normal control group ($p < 0.001$, chi-squared test). In the alendronate group, fractures were not observed at 7 and 14 days, but were observed in two out of nine replanted teeth at 21 days; the number of fractures was not significantly different from that in the normal control group ($p > 0.1$, chi-squared test).

Biomechanical properties of the periodontal ligament in replanted teeth

Load–deformation curves of the healing periodontal ligament — Figure 1 shows the load–deformation curves of the periodontal ligament in the normal control, control, and alendronate groups at 7, 14, and 21 days after replantation. Since eight out of nine replanted teeth in the control group at 21 days were fractured during mechanical testing, the biomechanical data were not shown in the graph. The rising parts of all the load–deformation curves were non-linear and almost sigmoid in shape. At 7 and 14 days, the load levels in the control and alendronate groups were markedly less than those in the normal control group at the same deformation levels. The load levels in the alendronate group were still less than those in the normal control group at 21 days.

Biomechanical measures of the healing periodontal ligament — Figure 2 shows changes in the maximum shear load (Fig. 2a), maximum shear deformation (Fig. 2b), tangent modulus (Fig. 2c), and failure energy in shear (Fig. 2d) of the periodontal ligament estimated from the load–deformation curves after replantation.

The maximum loads in the normal control group increased significantly during the experimental period ($p < 0.01$, ANOVA). In the control group, the mean value at 7 days after replantation decreased markedly ($p < 0.001$). Then, the mean values in the control group increased gradually from 7 to 14 days; the mean value at 14 days was not significantly different from the normal control. In the alendronate group, the mean value at 7 days decreased markedly ($p < 0.001$). Then, the mean values in the alendronate group increased gradually from 7 to 21 days (ANOVA, $p < 0.001$). The mean values were 62 and 67% of the normal control values at 14 and 21 days, respectively.

The maximum deformations in the normal control group did not change significantly during the experimental period. The mean values at 7 and 14 days in the control group were not significantly different from those of the normal controls. The mean value in the alendronate group decreased at 7 days, but increased gradually at 14 and 21 days, although the increases were not significant.

The tangent moduli in the normal control group increased gradually from 0 to 14 days (ANOVA, $p < 0.01$) and remained unchanged from 14 to 21 days. In the control group, the mean value at 7 days decreased ($p < 0.001$). Then, the mean values in

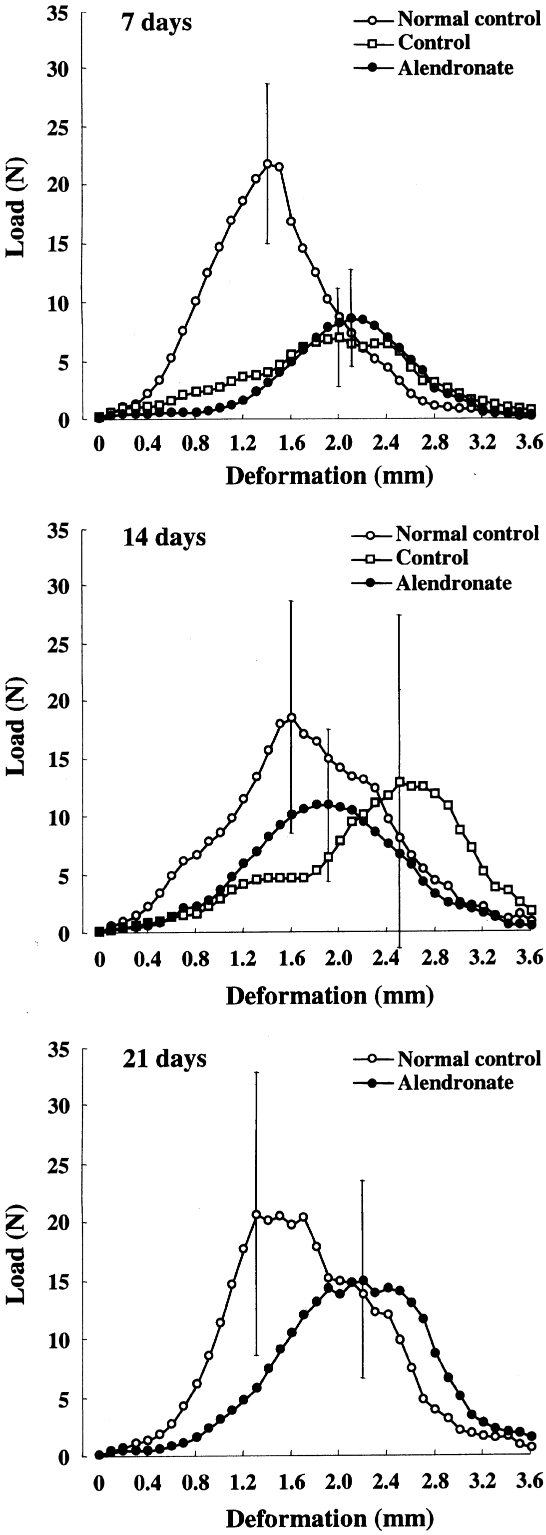

Fig. 1. Load–deformation curves for the periodontal ligament of the maxillary first molar at 7, 14, and 21 days after replantation in the normal control, control, and alendronate groups. The points on the graphs were plotted at intervals of 0.1 mm along the abscissa. Each point represents the mean of 6–10 rats. Vertical bars represent ± SD of the maximum shear load. The maximum shear load and maximum shear deformation for one specimen without fractures during mechanical testing in the control group at 21 days (Table 1) were 29.4 N and 1.91 mm, respectively (data not shown in the graph).

the control group increased from 7 to 14 days; the value at 14 days was not significantly different from the normal control value. In the alendronate group, the mean value at 7 days decreased markedly ($p < 0.001$). Then, the mean values in the alendronate group increased gradually from 7 to 21 days; the difference from the normal control was significant at 21 days ($p < 0.05$).

The failure energies in shear in the normal control group were unchanged from 0 to 14 days, but increased from 14 to 21 days. In the control group, the mean value at 7 days decreased markedly ($p < 0.001$). Then, the mean values in the control group increased gradually from 7 to 14 days. The mean value at 14 days was not significantly different from the normal control value. In the alendronate group, the mean value at 7 days also decreased markedly ($p < 0.001$). Then, the mean values in the alendronate group increased gradually from 7 to 21 days; the difference from the normal control value was significant at 21 days ($p < 0.01$).

Length of the mesial root in replanted teeth

Table 2 shows changes in the lengths of the mesial roots in the normal control, control, and alendronate groups. The mean values in the normal control group increased significantly during the experimental period (ANOVA, $p < 0.01$, 25% at 21 days). The mean values in the control group at 7 and 14 days did not differ from the value in the normal control group at day 0, and they were less than the respective normal control values at 7 and 14 days ($p < 0.05$ and $p < 0.001$); the values at 7 and 14 days were 92 and 82% of the normal control values, respectively. By contrast, the mean values in the alendronate group increased significantly from 0 to 21 days by 11% (ANOVA, $p < 0.05$), but they were less than the normal control values at 7, 14 ($p < 0.01$), and 21 ($p < 0.01$) days; the values at 7, 14, and 21 days were 97, 89, and 89% of the normal control values, respectively.

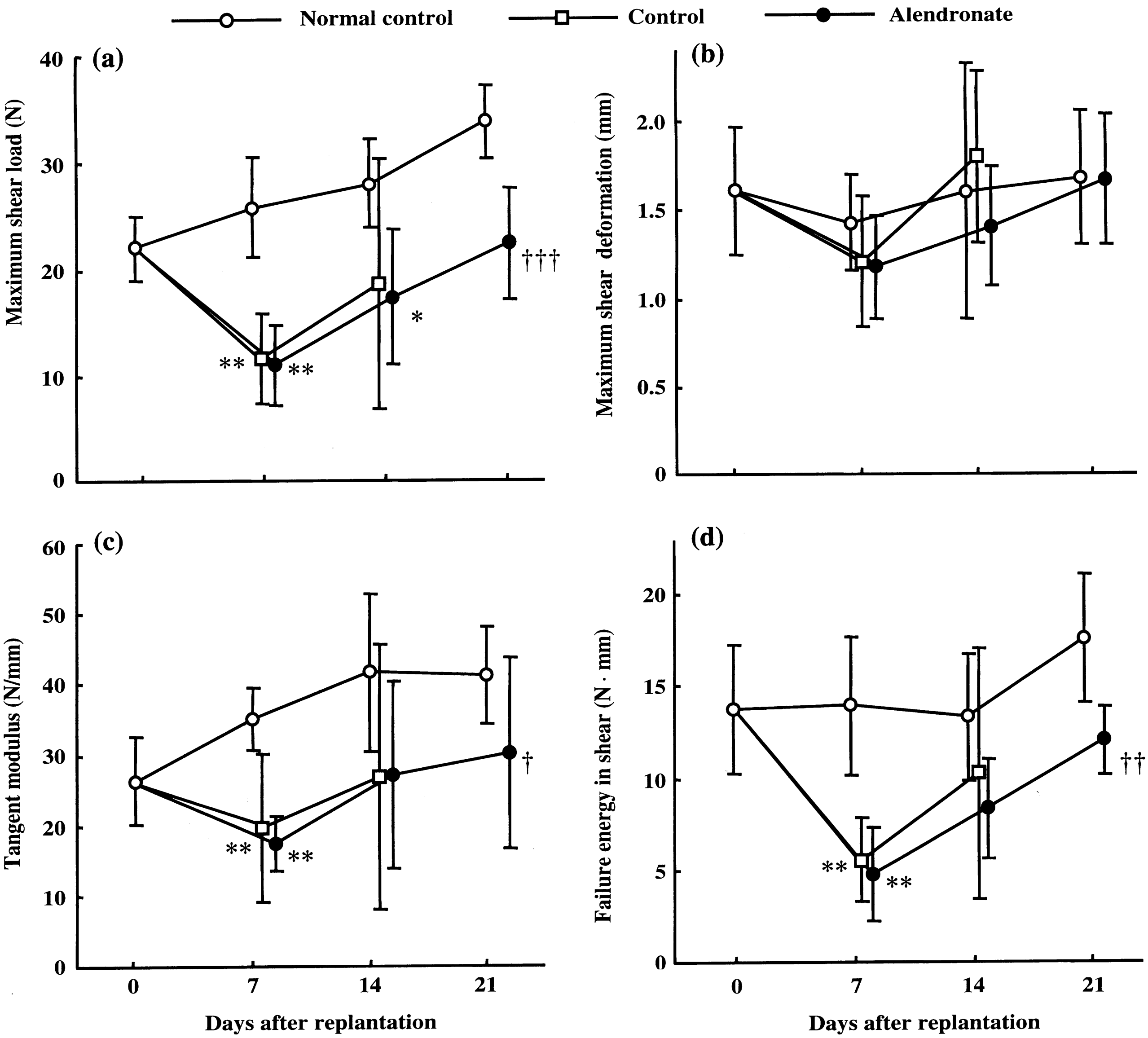

Fig. 2. Changes in the maximum shear load (a), maximum shear deformation (b), tangent modulus (c), and failure energy in shear (d) for the periodontal ligament of the maxillary first molar at 0, 7, 14, and 21 days after replantation in the normal control, control, and alendronate groups. Each point and vertical bars represents the mean ± SD of 6–10 rats. Significant differences from the respective normal control groups, $*p < 0.05$, $**p < 0.001$ (Scheffé's method). Significant differences from the normal control group at 21 days, $\dagger p < 0.05$, $\dagger\dagger p < 0.01$, $\dagger\dagger\dagger p < 0.001$ (t-test).

Table 2. Changes in the length of the mesial root of the maxillary first molar

Days after replantation	Length of mesial root (mm)		
	Normal control group	Control group	Alendronate group
0	1.99 ± 0.15 (9)	–	–
7	2.13 ± 0.13 (10)	1.97 ± 0.14* (9)	2.06 ± 0.12 (10)
14	2.38 ± 0.13 (9)	1.95 ± 0.12** (6)	2.11 ± 0.11** (10)
21	2.48 ± 0.14 (10)	–	2.21 ± 0.15†† (7)

Mean ± SD of the population is shown in each group.
Number of rats is shown in parentheses.
Significant differences from the normal control groups, $*p < 0.05$, $**p < 0.001$ (Scheffé's method), $\dagger\dagger p < 0.01$ (t-test).

Micro-computed tomography images of replanted teeth

Figure 3 shows mesio-distal images, reconstructed from micro-computed tomography, of the normal control (Figs 3a–c), control (Figs 3d–f), and alendronate (Figs 3g–i) groups at 7, 14, and 21 days after replantation. At 7 days, the periodontal spaces of the mesial roots in the control (Fig. 3d) and alendronate-pretreated (Fig. 3g) teeth appeared to be wider than those

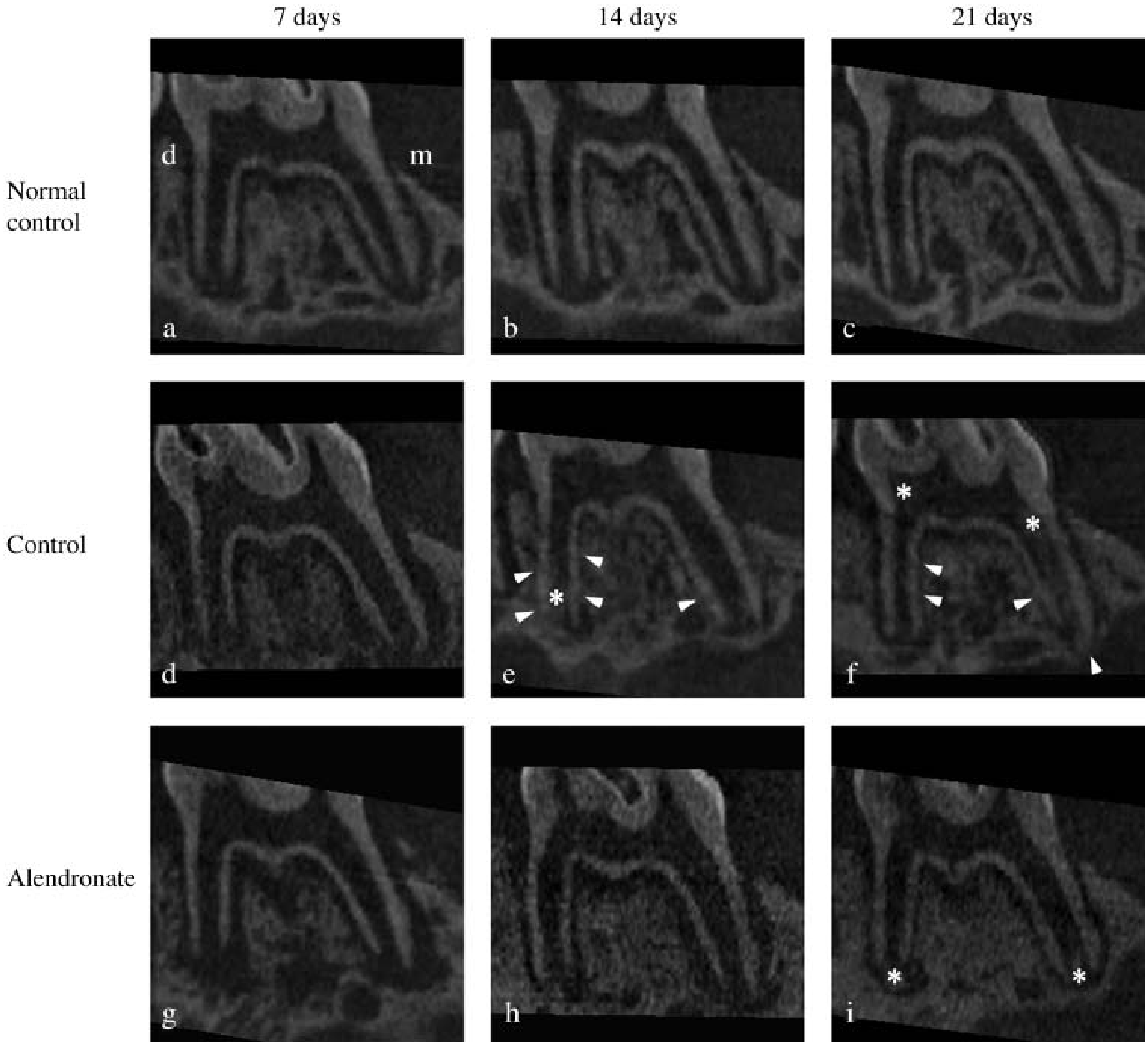

Fig. 3. Mesio-distal images of maxillary first molars reconstructed from micro-computed tomography. The specimens were obtained at 7, 14, and 21 days after tooth replantation. The left maxillary first molars were extracted, placed in saline (control; d, e, f) or saline containing 1 mM alendronate (g, h, i) for 5 min, and replanted into their original sockets. Normal control teeth (a, b, c) were not extracted. m, mesial side; d, distal side; arrowheads, ankylosis; asterisks, abnormal mineralized structures.

in the normal control teeth (Fig. 3a). The lengths of the mesial and distal roots of the normal control teeth at 14 (Fig. 3b) and 21 (Fig. 3c) days appeared to be longer than those of the control and alendronate-pretreated teeth. The control teeth at 14 (Fig. 3e) and 21 (Fig. 3f) days showed ankyloses around the mesial and distal roots. Abnormal mineralized structures were also seen within the dental pulp of the mesial and distal roots (Figs 3e and f). The alendronate-pretreated teeth at 14 (Fig. 3h) and 21 (Fig. 3i) days showed fewer ankylotic areas compared with the control teeth. Abnormal mineralized structures were only seen in the apical regions of the mesial and distal roots (Fig. 3i).

Light and polarized light microscopic observations

Figure 4 shows hematoxylin and eosin-stained sections of the normal control (Figs 4a and b), control (Figs 4c and d), and alendronate-pretreated (Figs 4e and f) teeth at 21 days. The bucco-distal (Figs 4a, c and e) and mesial (Figs 4b, d and f) roots of the maxillary first molars are indicated. The amount of cellular cementum on the mesial side of the mesial root appeared to be less in the control (Fig. 4d) and alendronate-pretreated (Fig. 4f) teeth compared with the normal control teeth (Fig. 4b). Bone-like structures were observed within the dental pulp of the distal and mesial roots markedly in the control teeth (Figs 4c and d) and to a lesser extent in the alendronate-pretreated teeth (Figs 4e and f). The normal arrangement of odontoblastic layers was not observed in the dental pulp of either the mesial or distal roots in the

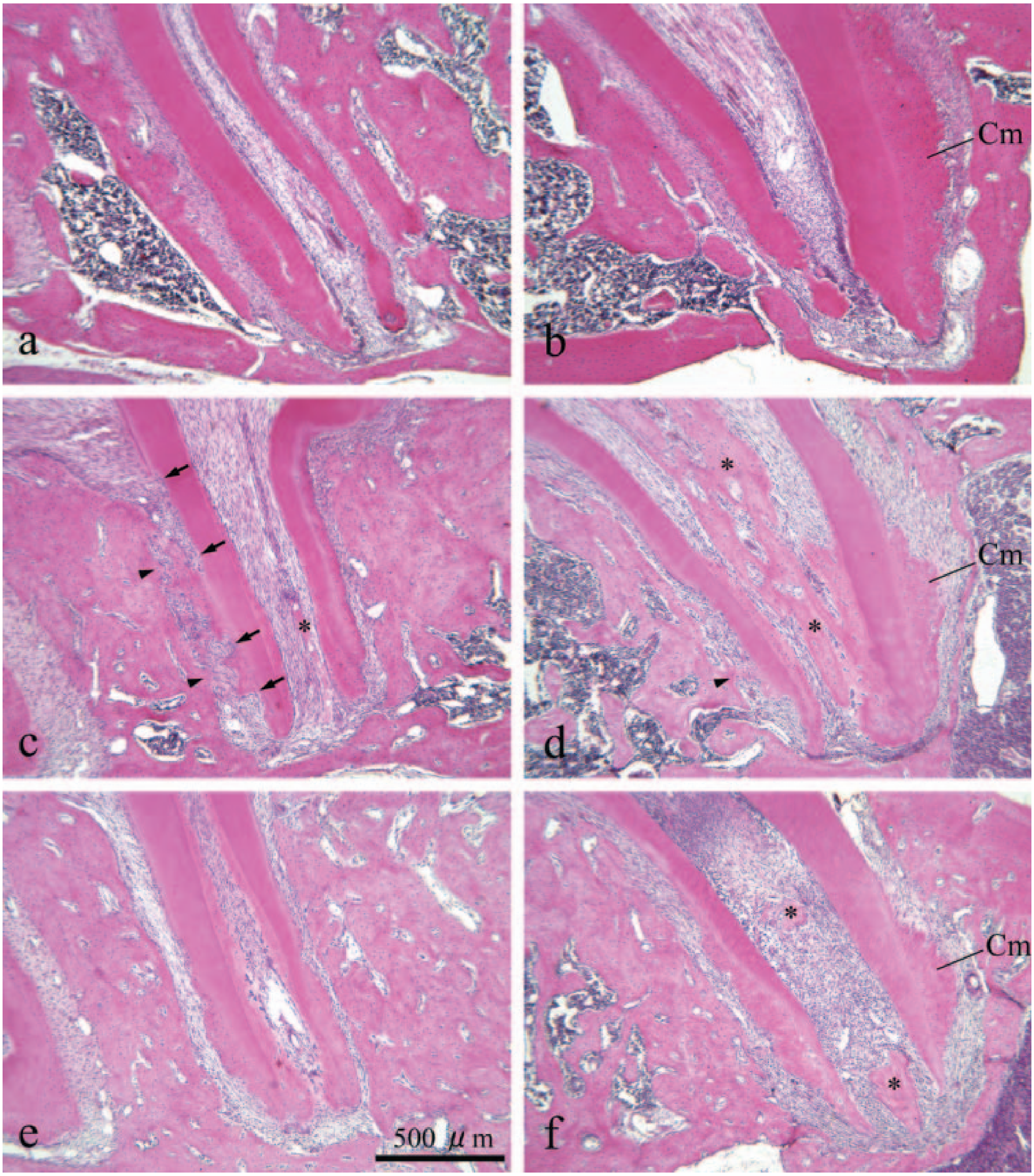

Fig. 4. Bucco-distal (a, c, e) and mesial (b, d, f) roots in mesio-distal sections of maxillary first molars at 21 days after replantation. Sections were obtained from a normal maxillary first molar (a, b), and replanted maxillary first molars after pretreatment with saline (c, d) and with 1 mM alendronate (e, f). Arrowheads, ankylosis; arrows, root resorption; asterisks, abnormal bone-like structures; Cm, cellular cementum. Hematoxylin and eosin.

control or alendronate-pretreated teeth (Figs 4c–f). Bony attachments were seen in the periodontal space of the distal sides of the distal and mesial roots in the control teeth (Figs 4c and d), but not in the alendronate-pretreated teeth (Figs 4e and f). Root resorptions were observed near the ankylotic areas on the distal side of the distal root in the control teeth (Fig. 4c).

Figure 5 shows ordinary and polarized light microscopic images of the periodontal ligament of the distal aspect of the mesial root in hematoxylin and eosin-stained sections of normal control (Figs 5a and b), control (Figs 5c and d), and alendronate-pretreated (Figs 5e and f) teeth. In the normal periodontal ligament, an oblique arrangement of birefringent

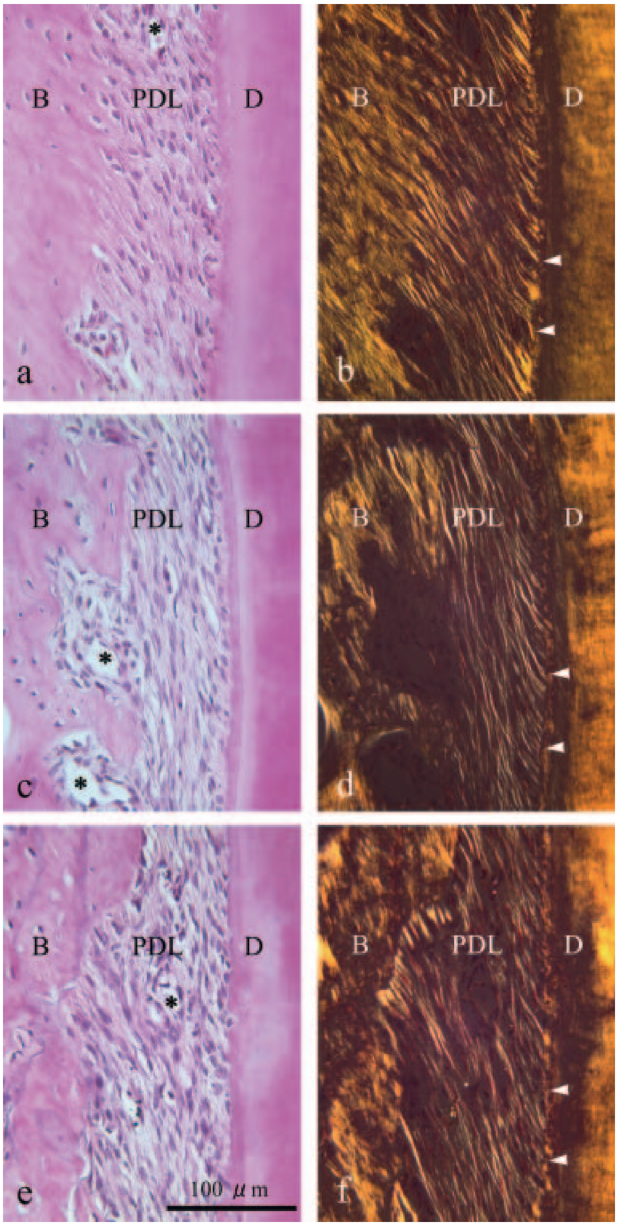

Fig. 5. High-power views of the mesial roots of maxillary first molars (Fig. 4) under ordinary (a, c, e) and polarized (b, d, f) light microscopes. Sections were obtained from a normal maxillary first molar (a, b), and replanted maxillary first molars after pretreatment with saline (c, d) and with 1 mM alendronate (e, f). B, bone; D, dentin; PDL, periodontal ligament; asterisks, blood vessels; arrowheads, root cementum. Hematoxylin and eosin.

collagen fiber bundles was seen between the root cementum and bone, and the fiber structures appeared to be well organized (Figs 5a and b). In the periodontal ligaments of the control (Fig. 5c) and alendronate-pretreated (Fig. 5e) teeth, the collagen fiber bundles appeared to be less organized than those of the normal control teeth (Fig. 5a). However, in the periodontal ligament of the alendronate-pretreated teeth, an oblique arrangement of birefringent collagen fiber bundles was seen between the root cementum and bone (Fig. 5f). Blood vessels were observed in the middle zone of the ligament and near the bone surfaces (Figs 5a, c and e).

Discussion

It is important to evaluate the effects of therapeutic agents on the wound healing of oral tissues using experimental animals (3, 6, 7, 9) that better model humans, such as do dogs and monkeys, before any clinical application of the agent. It is also desirable to use a relatively simple experimental model to determine the basic characteristics of a drug whenever possible (11). The rat molar model employed in the present study can serve both these purposes (4, 5, 16). The procedures of extraction and replantation of the rat molar are simple and easy. This model allows us to examine various aspects of the healing process in a sufficient number of animals to provide some statistical power to our findings. We previously established a method for the quantitative evaluation of the support function of the periodontal ligament by measuring the mechanical properties of the periodontal ligament in the rat molar (12).

Bisphosphonates are known to inhibit bone resorption and are currently used as therapeutic agents for bone diseases such as osteoporosis, Paget's disease, and cancer-related hypercalcemia (17). It is assumed that bisphosphonates mainly act through cellular mechanisms (e.g. inactivation of osteoclasts) other than the physicochemical mechanism of crystal dissolution, although the precise mechanisms of action remain to be elucidated (17). The topical administration of risendronate, a bisphosphonate, has been reported to have an inhibitory effect on root resorption in experimental orthodontic tooth movements (18). The topical administration of alendronate has also been reported to have beneficial effects on the healing of the cementum and may decrease the loss of root mass after replantation of dried dog teeth (7). It is clinically important to prevent the occurrence of root resorption because this generally worsens the prognosis of a replanted tooth (1, 2, 19, 20).

In the present study, there were marked fractures in the roots and/or bones during mechanical testing in the control group that occurred at 14 and

21 days after tooth replantation (Table 1). The fractures were effectively inhibited by topical pretreatment with alendronate. These fractures were considered to be mainly due to ankyloses between the molar roots and socket bones, as revealed by micro-computed tomography (Fig. 3) and histological (Fig. 4) analyses. It has been shown that administration of a bisphosphonate, 1-hydroxyethylidine-1,1-bisphosphonate, inhibits the mineralization of enamel and dentin in the rat incisor (21, 22) and the formation of the acellular cementum during root formation in the rat molar (23). In the present study, topical pretreatment with alendronate could have prevented the occurrence of ankyloses between the roots and bones, which was probably achieved via an inhibition of mineralization.

The mechanical properties of the healing periodontal ligament in the replanted teeth were restored similarly in the control and alendronate groups at 7 and 14 days (Fig. 2). Thereafter, the mechanical properties in the alendronate group continued to be restored until 21 days. The restoration rate (percentage of the normal control value) of the mechanical strength (67%) for the healing periodontal ligament in the alendronate group at 21 days was similar to that (62%) reported for luxated monkey teeth at 14 days (9) and greater than that (53%) reported for replanted rat incisors at 21 days (11). The restoration rates for the extensibility (98%) and toughness (68%) were greater than those for the monkey teeth (62 and 50%, respectively) and the rat incisors (85 and 52%, respectively). The most important functional parameters for characterizing healing wounds are considered to be the maximum and/or breaking energies (toughness), as external mechanical influences on the tissue are absorbed as energy (24). In that sense, pretreatment with alendronate may result in better restoration of the support function of the healing periodontal ligament in replanted teeth.

It has been shown that the restoration of the support function of the healing periodontal ligament in replanted rat incisors is closely related to the amount of reorganized collagen fibers and their functional reorientation (11). It has also been suggested that intermittent mechanical stimuli such as occlusal contacts play important roles in the reorganization and functional reorientation of collagen bundles (11). In the periodontal ligaments of the alendronate group, we observed an oblique arrangement of the fibers between the root cementum and bones, and fiber insertions into the bones (Figs 5e and f). By contrast, there appeared to be fewer fibers traversing between the root cementum and bones in the control group (Figs 5c and d) with ankylosis in the other areas of the periodontal ligaments. These findings suggest that the absence of ankylosis following pretreatment with alendronate might provide a better mechanical environment for the reorganization and functional reorientation of the collagen fibers in the healing periodontal ligament in replanted teeth.

The mean length of the mesial roots of the replanted teeth in the control group did not change after replantation. However, the mean length in the alendronate group increased by 11% between day 0 and day 21, although the values in the normal control group increased by 25% during this same period (Table 2). We assumed that the extraction and replantation inhibited the elongation of the roots, and that alendronate may have prevented this inhibition and restored the root elongation of the replanted teeth to some extent.

Bisphosphonates are orally or intravenously administered to patients in clinical settings (17), and the systemic administration of etidronate has been reported to have an inhibitory effect on heterotopic calcification (25, 26). However, it is known that the dose of etidronate that prevents heterotopic mineralization also inhibits normal calcification in bone, cartilage, dentin, and enamel (17). Tooth avulsion and replantation allow topical administration of a drug to the root surface (7). Therefore, we adopted a topical administration of alendronate.

It has been reported that the pretreatment of roots with dexamethasone enhanced healing and resulted in fewer resorptions after 12 weeks in replanted dog teeth (27) and after 3 weeks in replanted rat molars (28). However, the dexamethasone-treated group exhibited more bone ankylosis than the non-treated group in replanted rat molars (28). In the present study, the pretreatment with alendronate effectively inhibited ankylosis. Therefore, further studies are required to better understand the roles and mechanisms of bisphosphonates and anti-inflammatory drugs in the restoration processes of avulsed teeth.

References

1. Andreassen JO, Andreassen FM. *Textbook and color atlas of traumatic injuries to the teeth*, 3rd edition. Copenhagen: Munksgaard, 1994.
2. Tsukiboshi M. Wound healing in transplantation and replantation. In: Tsukiboshi, M, ed. *Autotransplantation of teeth*. Chicago: Quintessence Publishing Co, 2001: 21–55.
3. Löe H, Waerhaug J. Experimental replantation of teeth in dogs and monkeys. *Arch Oral Biol* 1961;**3:**176–184.
4. Kvinnsland I, Heyeraas KJ, Byers MR. Regeneration of calcitonin gene-related peptide immunoreactive nerves in replanted rat molars and their supporting tissues. *Arch Oral Biol* 1991;**36:**815–826.
5. Nishioka M, Shinya T, Ueno K, Suda H. Tooth replantation in germ-free and conventional rats. *Endod Dent Traumatol* 1988;**14:**163–173.
6. Selvig KA, Bjorvatn K, Claffey N. Effect of stannous floride and tetracycline on repair after delayed replantation of root-planed teeth in dogs. *Acta Odontol Scand* 1990;**48:**107–112.
7. Levin L, Bryson EC, Caplan D, Trope M. Effect of topical alendronate on root resorption of dried replanted dog teeth. *Dent Traumatol* 2001;**17:**120–126.
8. Gottrup F, Andreasen JO. Wound healing subsequent to injury. In: Andreasen, JO, Andreasen, FM, eds. *Textbook and color atlas of traumatic injuries to the teeth*, 3rd edition. Copenhagen: Munksgaard, 1994: 13–76.
9. Mandel U, Viidik A. Effect of splinting on the mechanical and histological properties of the healing periodontal ligament in the vervet monkey (*Cercopithecus aethiops*). *Arch Oral Biol* 1989;**34:**209–217.
10. Fukui T, Yamane A, Komatsu K, Chiba M. Restoration of mechanical strength and morphological features of the periodontal ligament following orthodontic

retention in the rat mandibular first molar. *Eur J Orthod* 2003;**25:**167–174.

11. Shinohara J, Shibata T, Shimada A, Komatsu K. The biomechanical properties of the healing periodontium of replanted rat mandibular incisors. *Dent Traumatol* 2004;**20:**212–221.
12. Chiba M, Ohkawa S. Measurement of the tensile strength of the periodontium in the rat mandibular first molar. *Arch Oral Biol* 1980;**25:**569–572.
13. Mandel U, Dalgaard P, Viidik A. A biomechanical study of the human periodontal ligament. *J Biomech* 1986;**19:**637–645.
14. Yamazaki Y. Effects of destructive periodontitis, induced by diet, on the mechanical properties of the periodontal ligament of the mandibular first molar in golden hamsters. *J Periodont Res* 1992;**27:**149–158.
15. Kinoshita Y, Tonooka K, Chiba M. The effect of hypofunction on the mechanical properties of the periodontium in the rat mandibular first molar. *Arch Oral Biol* 1982;**27:**881–885.
16. Ohshima H, Nakakura-Ohshima K, Yamamoto H, Maeda T. Alteration in the expression of heat shock protein (Hsp) 25-immunoreactivity in the dental pulp of rat molars following tooth replantation. *Arch Histol Cytol* 2001;**64:**425–437.
17. Fleisch H. *Bisphosphonates in bone disease*. San Diego: Academic Press, 2000.
18. Igarashi K, Adachi H, Mitani H, Shinoda H. Inhibitory effect of the topical administration of a bisphosphonate (risendronate) on root resorption incident to orthodontic tooth movements in rats. *J Dent Res* 1996;**75:**1644–1649.
19. Newman HN. Trauma and the periodontal ligament. In: Berkovitz, BKB, Moxham, BJ, Newman, HN, eds. *The Periodontal ligament in health and disease*. 2nd edition. London: Mosby-Wolfe, 1995: 315–340.
20. Berggreen E, Sae-Lim V, Bletsa A, Heyeraas KJ. Effect of denervation on healing after tooth replantation in the ferret. *Acta Odontol Scand* 2001;**59:**379–385.
21. Izumi T. Effects of 1-hydroxyethylidene-1,1-bisphosphonate (HEBP) on the unimpeded eruption rate and the dental hard tissue formation in rat mandibular incisor. *Nippon Yakwigaku Zasshi* 1989;**93:**75–87.
22. Fuangtharnthip P, Yamada Y, Takagi Y, Ohya K. Autoradiographic investigation of the effect of 1-hydroxyethylidene-1,1-bisphosphonate on matrix protein synthesis and secretion by secretory ameloblasts in rat incisors. *Arch Oral Biol* 2000;**45:**495–506.
23. Alatli I, Li J, Hammarström L. Ultimate tensile strength of PDL of molars in rats after 1-hydroxyethylidene-1,1-bisphosphonate injections. *J Dent* 1997;**25:**313–319.
24. Quirinia A, Viidik A. Freezing for postmortal storage influences the biomechanical properties of linear skin wounds. *J Biomech* 1991;**24:**819–823.
25. Fleisch HA, Russell RG, Bisaz S, Muhlbauer RC, Williams DA. The inhibitory effect of phosphonates on the formation of calcium phosphate crystals in vitro and on aortic and kidney calcification in vivo. *Eur J Clin Invest* 1970;**1:**12–18.
26. Ruml LA, Dubois SK, Roberts ML, Pak CY. Prevention of hypercalciuria and stone-forming propensity during prolonged bedrest by alendronate. *J Bone Miner Res* 1985;**10:**655–662.
27. Sae-Lim V, Metzger Z, Trope M. Local dexamethasone improves periodontal healing of replanted dogs' teeth. *Endod Dent Traumatol* 1998;**14:**232–236.
28. Keum K-Y, Kwon O-T, Spängberg LS, *et al*. Effect of dexamethasone on root resorption after delayed replantation of rat tooth. *J Endod* 2003;**29:**810–813.