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Morphological changes in the rat periodontal ligament and its vascularity after experimental tooth movement using superelastic forces

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SUMMARY The aim of this study was to statistically assess the morphological changes of the rat periodontal ligament (PDL) and its vascularity in relation to varied magnitudes of superelastic force in experimental tooth movement using nickel–titanium (NiTi) alloy wire. Forces of 0.8, 1.6, 4, 8, and 18 g were applied to the upper first molars of five groups of 10-week-old male Wistar rats (300–320 g) for 1, 7, 14, 21, and 28 days. A control group with no orthodontic appliance application was assessed in accordance with the five experimental periods. The specimens were observed under light microscopy, processed by computer imaging, and analysed statistically with Tukey's HSD non-parametric test.

One day after the start of the experiment, a few blood vessels could be seen in the compressed PDL with forces of 0.8 and 1.6 g. The cross-sectional areas of blood vessels (CAV) and periodontal ligament (CAPL) in the experimental groups where a force of over 4 g was applied were significantly smaller than those where 0.8 and 1.6 g forces were used, and in the control group. On day 7, large CAV were seen in the 1.6, 4, and 8 g groups. On day 28, the 8 and 18 g groups showed significantly larger CAPL than the 0.8, 4 g, or control groups.

The findings suggest that a light continuous force, under 1.6 g, maintains the vascular structure during experimental tooth movement. In contrast, a heavy continuous force over 4 g causes the vascular structure to be absent in the early stages of tooth movement, but a dynamic regeneration of the PDL with vascularity and expansion follows.

Introduction

Superelastic force, as a property of nickel–titanium (NiTi) alloy wire, is characterized by a light continuous force with a long range of activation (Miura *et al.*, 1986; von Fraunhofer *et al.*, 1993, Wilkinson *et al.*, 2002). With this force, teeth can be moved effectively and smoothly resulting in optimal tooth movement in humans (Miura *et al.*, 1988; Iwasaki *et al.*, 2000) and rats (Suzuki *et al.*, 2006). However, Fernandes *et al.* (1998) reported that the level of pain/discomfort increased in the initial phase of tooth movement when using a superelastic NiTi aligning archwire. To date, it has not been possible to determine whether such a superelastic mechanism enables biologically safe and efficient tooth movement. It is therefore speculated that superelastic force leads to efficient tooth movement, but some pathological damage in the periodontal ligament (PDL). A light force influences the vasculature of the PDL (Gianelly, 1969; Gaengler and Merte, 1983; Noda *et al.*, 2000), and a heavier force, partial or total occlusion of vessels (Khouw and Goldhaber, 1970), resulting in degeneration or necrosis of the PDL (Macapanpan *et al.*, 1954; Reitan, 1960; Nakamura, 1967; Kvam, 1969; Azuma, 1970; Rygh, 1972, 1974; Vandevska-Radunovic *et al.*, 1994; Noda *et al.*, 1997).

To maintain the biological activity of compressed PDL, a compressed condition without obstructing blood circulation should be considered during tooth movement. A previous study (Noda *et al.*, 2000) indicated that PDL tissue responded favourably to a light continuous force of 0.8–1.6 g (3.21–6.24 g/cm^2), and no degeneration appeared in the compressed region. In contrast, a heavy continuous force over 4 g (16.22 g/cm^2) compressed the PDL to a greater extent, resulting in degenerating tissue without vascular structures. Tooth movement in the heavy force group was significantly greater than that in the light force group. As described by Burstone (1985), an optimal stress level that basically maintains the vitality of the tissue throughout its length should be used. It is important to know the morphological changes of the PDL and its vascularity that are assumed to reflect PDL compression and blood circulation in relation to various continuous forces.

The aim of the present study was to statistically assess the morphological changes of the PDL and its vascularity in relation to various magnitudes of superelastic force using NiTi wires during experimental tooth movement.

Materials and methods

The experimental protocols were approved by the Institutional Animal Care and Use Committee of Tsurumi University, and the experiment was carried out in accordance with the Guidelines for Animal Experimentation.

Adjustment of the experimental appliance and tooth movement

An orthodontic appliance, with two NiTi wire springs (Sentalloy 0.016 inch round wire; Tomy International Co. Ltd., Tokyo, Japan) (Figure 1a,b), was used to move the maxillary first molars of 10-week-old male Wistar rats (90 rats, body weight range: 300–320 g), under pentobarbital sodium anaesthesia. Five force magnitudes of 0.8 (force range: 0.73–0.86 g), 1.6 (1.54–1.75 g), 4 (3.73–4.27 g), 8 (7.61–8.35 g), and 18 g (17.64–18.40 g) (Noda *et al.*, 2000) (Figure 2a,b) were applied by reducing the NiTi wire with a carborundum point under water cooling using a system which measured the character of the loading (g)—distortion (mm) of each wire spring (Figure 2a) at a temperature of approximately 37°C. The system comprised an electronic balance (minimum resolution: 0.001 g), a micrometer (minimum resolution: 0.05 mm), and a locking device for the wire-spring appliance. When moved downward, the tip pressed the wire spring, the spring was distorted, and the pressure displayed on the balance. The measurement was carried out at intervals of 0.1 mm distortion, adjusting the maximum pressure at 1.0 mm distortion for each experimental force magnitude.

The experimental periods were 1, 7, 14, 21, and 28 days after the start of tooth movement. To compare and evaluate the response of the PDL tissue against very light mechanical stimulation with a force of 0.8 g, control groups were defined as rats without an orthodontic appliance. Three rats were allocated to each of the five experimental periods in the five experimental groups and one control group, 90 rats in total.

Measurement of the distance moved

A calliper was utilized to measure the distance between the upper right and left first molars in each experimental and control group on days 1, 7, 14, 21, and 28. The distances measured in the experimental groups were corrected by subtracting the control distance to eliminate growth, and divided by two to obtain the unilateral result.

Histological procedure

After tooth movement, all rats were perfused with 2.5 per cent glutaraldehyde fixative (0.1 M phosphate buffer) for 10

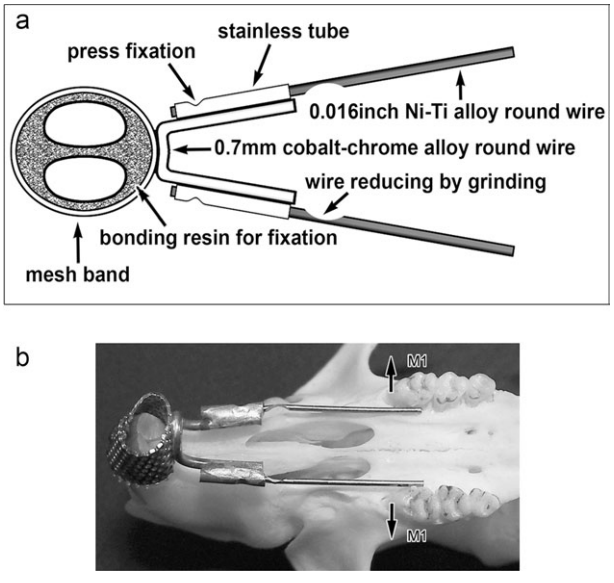

Figure 1 (a) Illustration of the orthodontic appliance applying a super elastic force. The appliance consists of a mesh band, 0.7 mm cobalt–chrome round wire, two short stainless tubes, and a 0.016 inch nickel–titanium (NiTi) alloy round wire. The parts without a NiTi wire were assembled with silver solder. The wire was inserted into the tube and fixed by pressure. The orthodontic force was adjusted by grinding and reducing the wire under water cooling. (b) An image of the appliance on a rat dry skull. Two wire springs are applied to the upper first molars (M1) on both sides, achieving tooth movement in a buccal direction (arrow).

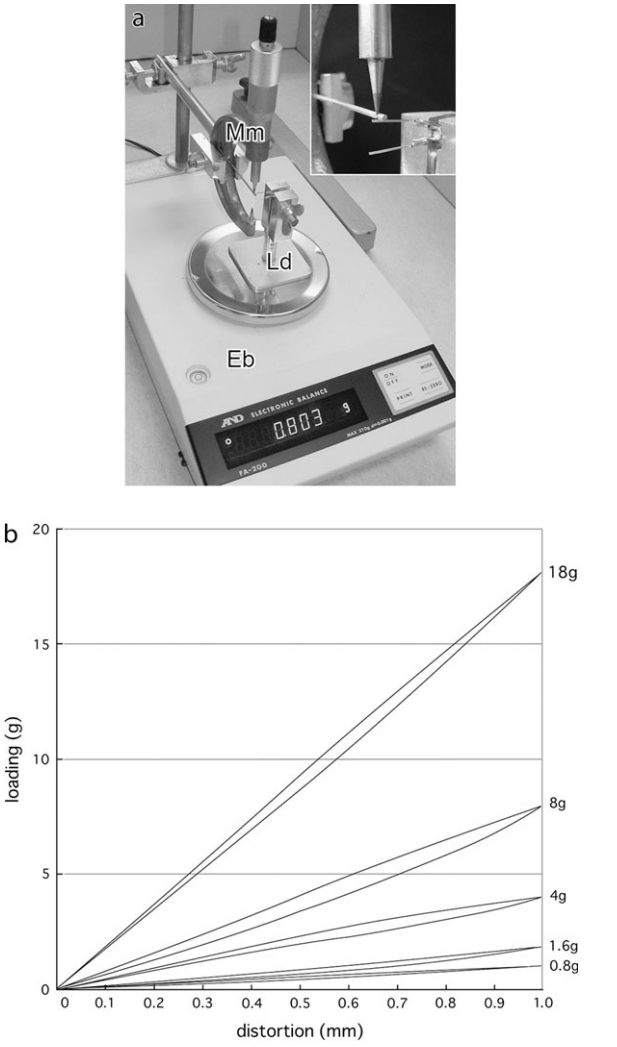

Figure 2 (a) The system used to measure the loading (g)–distortion (mm) of each wire spring. The system consists of an electronic balance (Eb, minimum resolution: 0.001 g), a micrometre (Mm, minimum resolution: 0.05 mm), and a locking device (Ld) for the wire spring. Insert: A magnified image of the measuring point, showing the relationship between the tip of the micrometre and wire spring. (b) Characteristics of loading (g)–distortion (mm) curve in nickel–titanium springs incorporated into orthodontic appliances used in each experimental group.

minutes through the ascending aorta under deep anaesthesia. The maxillae were resected and trimmed around the upper first molars. The trimmed tissue blocks were decalcified by immersion in 5 per cent ethylenediaminetetraacetic acid–2Na (7 per cent sucrose, pH 7.4, 4°C) for 10 days. After washing overnight with 0.1 M phosphate buffer under cold conditions, 90 μm thick serial sections of the tissue blocks were prepared perpendicular to the root axis using a Vibratome (Series-1000; Technical Products International, Inc., St Louis, Missouri, USA). After post-fixation with 1.0 per cent osmium tetroxide for 1 hour, the sections were dehydrated with a graded series of ethanol and embedded in epoxy resin (Epok 812; Ouken Syoji Co. Ltd., Tokyo, Japan). Serial semi-thin sections, approximately 15 sections, 2.5–3 μm thick were cut from the resin-embedded thick sections using a diamond knife and stained with toluidine blue. Finally, a section including the maximum compressed region and showing the most suitable condition for image processing was selected from the serial sections.

Image processing and statistical analysis

An area measuring 500 × 1000 μm^2, including the maximum compressed region near the centre, was selected on the semi-thin section for light microscopic examination (Figure 3a,b). Six sections obtained from different specimens were recorded on reversal film (Vanox; Olympus Corporation, Tokyo, Japan) in each experimental and control group. After scanning of the film (Polascan 35; Nippon Polaroid, Tokyo, Japan), the data were processed and analysed twice by a real-time image analyser (Luzex-FS; Nireco Co. Ltd., Tokyo, Japan) (Figure 3c), and the average of the two readings was used as original data (Table 1). The data for vascular and PDL areas were analysed and assessed using Tukey's HSD non-parametric test with the Statistical Package for Social Sciences version 6.1 for Mac (SPSS Japan Inc., Tokyo, Japan).

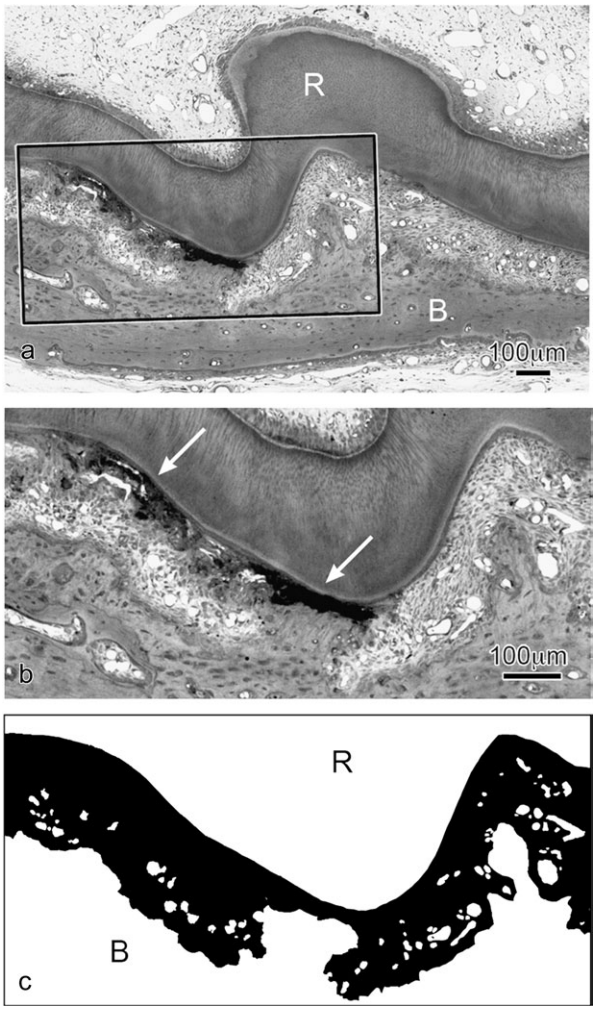

Figure 3 Images of the measurement area with toluidine blue staining and with computer processing. (a) A measurement area of 500 × 1000 μm^2 including the maximum compressed region, surrounded by a black line, was selected on the buccal side of the periodontal ligament (PDL). R: dental root; B: bone (b) A higher magnification of the measurement area shown in Figure 3a. Two degenerating regions with intense staining (arrows) can be seen on the compressed side. (c) Extracted processing image of the PDL and vascular cross-sectional areas. Black-coloured area shows the PDL cross-section and numerous white blood vessels.

Results

Amount of tooth movement

All groups showed a steep increase in the amount of tooth movement from the start of the experiment to day 1 (Figure 4). The distance in the 0.8 g group tended to plateau after day 1, while that in the 1.6 g group increased gradually until day 21. On the other hand, further increases were seen in experimental groups over 4 g after day 14. The longest distance was 0.675 mm in the 8 g group, with values decreasing in the following order: 18, 4, 1.6, and 0.8 g groups, which showed the least distance, 0.063 mm on day 28.

Computer-processed images of the PDL and its vascular supply in the measured area

In selected images of the PDL, the blood vessels were revealed as small white circles scattered in the PDL for all experimental periods (Figure 5). In the 0.8 and 1.6 g groups, the PDL was narrower in the maximally compressed region on day 1, with only a few blood vessels present. Conversely, PDL images in the 4, 8, and 18 g groups showed marked narrowing between the bone and root surface, and no blood vessels could be seen in this area on day 1. The outline of the bone surface in these groups was ruffled on days 7 and 14, indicating severe undermining bone resorption.

Total cross-sectional area of blood vessels (CAV) in PDL

The median values of CAV over time (Figure 6a, Table 1) in the 0.8 g and control groups showed an increase and decrease, respectively, with narrow variations. No significant difference was noted in these groups during the experimental

Table 1 Median and maximum–minimum values of the total cross-sectional areas of the blood vessels and periodontal ligament (PDL) without blood vessels in the experimental and control groups.

		Total cross-sectional areas of blood vessels (μm²)			Cross-sectional areas of PDL without blood vessels (μm²)		
		Median	Max	Min	Median	Max	Min
0.8 g	Day 1	4504	6061	1269	155854	173873	105709
	Day 7	5503	13773	2007	168405	215886	123262
	Day 14	5221	7544	3377	182425	205235	147080
	Day 21	7936	11254	1072	188717	217969	149092
	Day 28	6380	9669	3935	161975	180055	132385
1.6 g	Day 1	4462	5668	3314	137752	174380	127366
	Day 7	9679	14583	7343	184378	225911	139131
	Day 14	6529	7980	4825	179093	184716	159333
	Day 21	5467	18035	3240	220752	251067	170604
	Day 28	9724	10835	9080	190926	236563	159576
4 g	Day 1	760	1506	272	84769	96817	56825
	Day 7	6690	11575	3769	136504	236168	120751
	Day 14	7178	8884	1141	160064	188849	113522
	Day 21	5944	9738	4795	160620	222899	112212
	Day 28	8178	15042	6605	163411	214089	139537
8 g	Day 1	944	1185	109	90389	106909	87007
	Day 7	10931	18688	4390	184846	205738	128079
	Day 14	12689	14655	4686	203969	241170	165491
	Day 21	6102	7812	3289	184982	216543	123218
	Day 28	8642	19699	6926	237717	321086	179037
18 g	Day 1	1485	2141	252	77842	93281	73322
	Day 7	2813	3719	1156	113292	154272	77230
	Day 14	6216	8430	1847	173157	239921	132440
	Day 21	7824.5	14306	6114	217889	251591	156859
	Day 28	9849	12069	6040	242809	305486	188831
Control	Day 1	6415	7699	4736	200636	208726	170296
	Day 7	5161	5161	3022	193381	193381	150558
	Day 14	5957	6662	4741	175851	201494	148084
	Day 21	4827.2	5704	4351	168819	208953	154049
	Day 28	6574	8504	4934	179723	193437	149778

periods (Figure 7). The 1.6 g group showed a steep increase in CAV between days 1 and 7, with a peak on day 7. CAV on day 1 was markedly smaller in the experimental groups over 4 g (Figure 6a, Table 1), and many significant differences were seen between day 1 and the other experimental periods in these groups (Figure 7a). The 4 g group showed two peaks on days 14 and 28, and steep increases were observed between days 1 and 7 and between days 21 and 28 (Figure 6a). The 8 g group showed three peaks on days 7, 14, and 28, with steep increases noted between days 1 and 7 and between days 21 and 28, similar to the 4 g groups (Figure 6a). The 18 g group showed a gradual increase during the experimental period, with significant differences between days 1 and 14, day 21 or 28, and between days 7 and 21 or 28 (Figure 7a).

Comparing the median values of CAV on day 1 (Figures 6a, 8a and Table 1), all experimental groups showed significantly smaller values than the controls. The experimental groups over 4 g had significantly ($P < 0.01$) smaller CAV than the 0.8 and 1.6 g groups (Figure 8a). The largest CAV was noted in the 8 g group on day 14. There was no significant difference among experimental and control groups on days 21 and 28 (Figure 8a).

Cross-sectional area of PDL (CAPL) without blood vessels

The median values of the CAPL in the 0.8 g and control group showed small variations with no significant difference during the experimental periods. The 1.6 g group showed a pattern of a narrow increase and decrease. The CAPL on day 1 was significantly smaller than that on day 21 or 28. The CAPL on day 1 in the experimental groups over 4 g was markedly smaller (Figure 6b), and many significant differences were seen between day 1 and the other experimental periods (Figure 6b), resembling the results of CAV. The 4 g group showed a marked increase between days 1 and 7 and a plateau after day 7 (Figure 6b). The 8 g group showed marked increases between days 1 and 7 and between days 21 and 28, with two peaks on days 14 and 28 (Figure 6b). In the 18 g group, there was a gradual increase during the experimental period, as seen in the results for CAV, and significant differences ($P < 0.01$) were noted between days 1 and 7 and 14, 21, and 28 (Figure 7b).

Comparing the median values of CAPL in the experimental periods (Figures 6b, 8a and Table 1), all experimental groups showed significantly smaller values than the controls on day 1. The experimental groups over 4 g had significantly

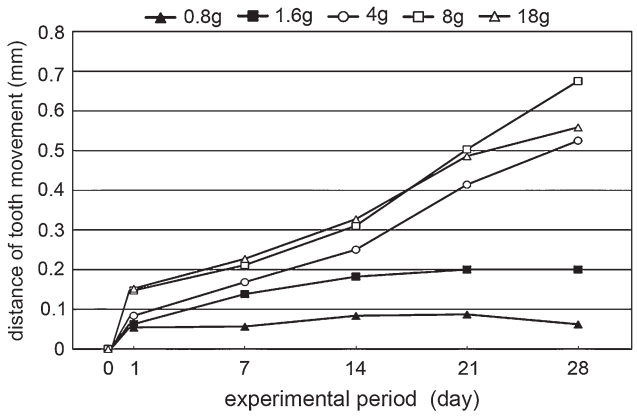

Figure 4 Graph of tooth movement in the experimental groups.

smaller CAPL than the 0.8 and 1.6 g groups on the same day (Figure 8). The CAPL of the 18 g group was significantly smaller than that of the 1.6 and 8 g ($P < 0.05$) and control ($P < 0.01$) groups on day 7. There was no significant difference among the experimental and control groups on days 14 and 21 (Figure 8). The 8 and 18 g groups showed larger CAPL than the 0.8, 4 g, and control groups on day 28. The largest CAPL in the experimental period was in the 18 g group on day 28 (Table 1).

Discussion

Comparison of tooth movement with force magnitude applied

Reitan (1960) reported that there are initial and secondary periods during tooth movement when using a continuous force of 200 g. During the initial period, sudden compression of the PDL occurs, where the tissue shows necrosis or degeneration, and the distance moved is within the PDL width. All experimental groups in the present study showed a steep increase in tooth movement on day 1, and this change may be regarded as the initial period. The minimum force magnitude used in this study was 0.8 g, and it is suggested that PDL responds to even a very light continuous force. Based on previous findings that the root surface area of the upper first molar in Wistar rat is 24.91 mm^2 (Noda *et al.*, 2000), a 0.8 g force corresponds to 3.21 g/cm^2 of root surface area and approximately 9 g in a human upper canine (Jepsen, 1963). The distances moved in the experimental groups within a force magnitude of 1.6 g were obviously shorter than those in groups where a force of over 4 g was applied during the experimental period.

PDL and its vascular response

Blood vessels in the PDL respond differently depending on the force magnitude (Gianelly, 1969; Khouw and Goldhaber, 1970; Gaengler and Merte, 1983). A heavy force causes severe damage to the compressed PDL,

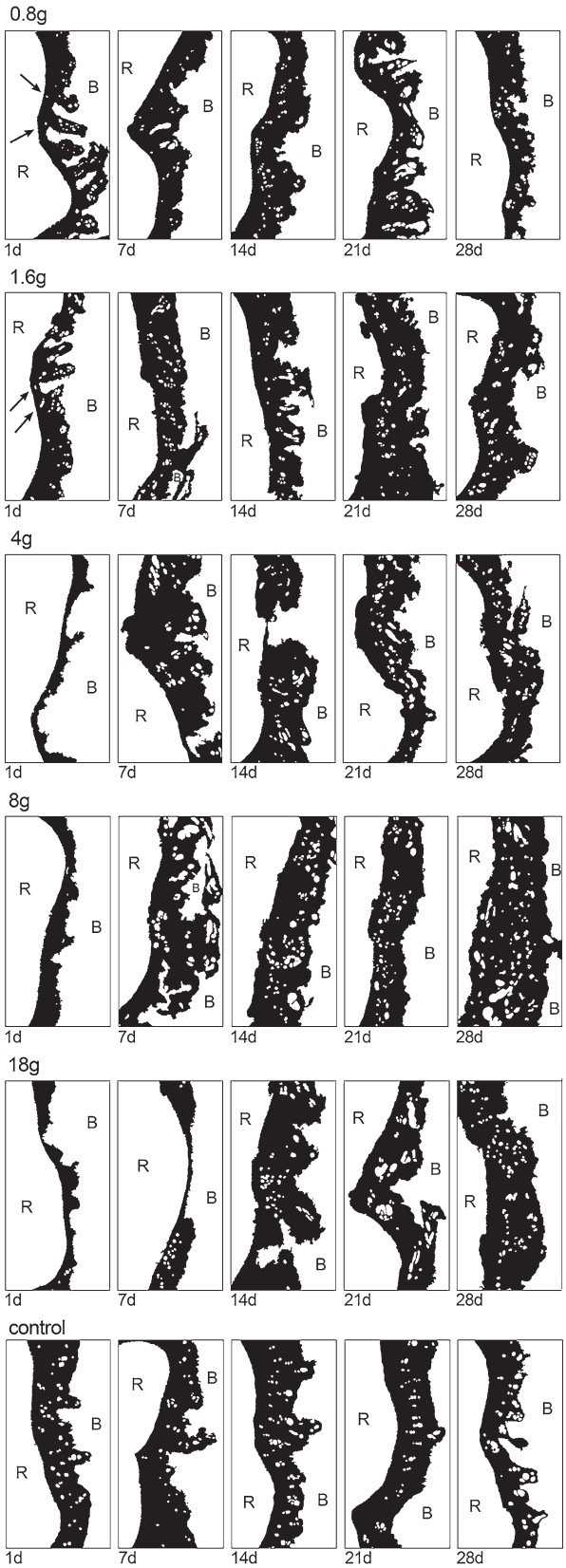

Figure 5 Computer-processing images of the experimental and control groups for the cross-sectional areas of the periodontal ligament and its blood vessels at 1, 7, 14, 21, and 28 days after tooth movement.

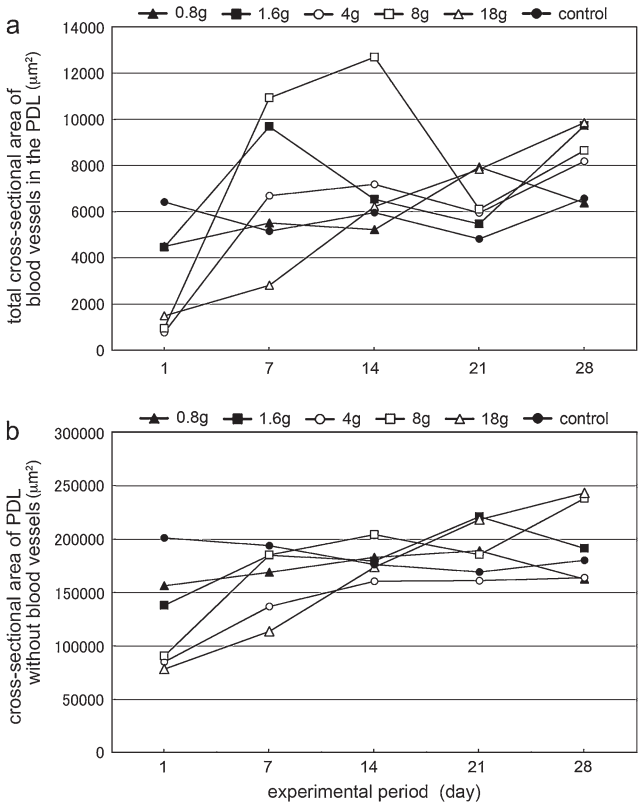

Figure 6 Graphs showing the median values of the total cross-sectional areas of blood vessels (a) and the periodontal ligament without the vessels (b) in the experimental and control groups during the experimental periods.

resulting in vascular deformation or degeneration (Rygh, 1972; Nakamura *et al.*, 1986). On the other hand, the response of PDL to light forces is unclear. On day 1, the CAV in the experimental groups with a force magnitude less than under 1.6 g was significantly larger than the CAV in groups over 4 g (Table 1). In a previous study (Noda *et al.*, 2000), degenerating tissue was seen at the maximally compressed region of the PDL in experimental groups in which a force magnitude of over 4 g was applied (16.2 g/cm^2), while degeneration was not observed in the experimental groups under 1.6 g (6.4 g/cm^2), suggesting that the magnitude of the threshold force causing vascular resistance exists between 1.6 and 4 g. Guyton and Hall (2000) stated that capillary blood pressure is a fundamental factor which affects the vascular active transport system as well as maintaining the vascular form, and the pressure measured using the isogravimetric method, considered as a functional pressure, is 17 mmHg (23 g/cm^2) in humans. The capillary blood pressure of the rat PDL has not yet been determined. Kristiansen and Heyeraas (1989) reported that the interstitial fluid pressure in rat PDL is 15.2 mmHg. Based on that finding and the physiological fact that capillary pressure is generally higher than interstitial fluid pressure (Guyton and Hall, 2000), it can be speculated that the capillary pressure is not less than 15.2 mmHg (21 g/cm^2) in rat PDL. This value is close to the result reported by Schwarz (1932), who found that an orthodontic force which brought the capillary pressure below 20–26 g/cm^2 would be biologically acceptable for tipping movement of premolars in beagle dogs.

Both CAV and CAPL in the 0.8 g group were significantly smaller than those in the control on day 1. This result accords with the steep increase in tooth movement in the 0.8 g group on day 1 and indicates that PDL tissue deforms even with a very light continuous force such as 0.8 g (3.2 g/cm^2). Kawarizadeh *et al.* (2003) reported that the Young's modulus of PDL elasticity in the initial phase of tooth mobility is 0.15 MPa (1.53 g/cm^2) in the lower first molar of Wistar rats. It is speculated that tooth movement with a force of 0.8 g occurs in the range of initial tooth mobility in the first molars of Wistar rat.

As seen in Table 1, the median values of CAV in the experimental groups, except the 18 g group, were larger than that of the controls on day 7. Rygh (1972) reported that buccally compressed PDL exhibited a reparative phase with proliferation of new vascular elements after an experimental period of more than 7 days when using a wire-spring appliance. Vandevska-Radunovic *et al.* (1994) noted that maximum blood flow was seen in the upper first molar of Wistar rats on day 7 after tooth movement using a mesially tensioned coil spring. It is speculated that substantive revascularization might appear at the compressed region on day 7 after tooth movement, regardless of the appearance of degenerating tissue. This could be interpreted in such a way that an excessive continuous force of 18 g (72.3 g/cm^2) may delay the revascularization, as the PDL is severely compressed, with CAV less than the control on day 7.

In the present study, the 8 g group demonstrated the greatest tooth movement, followed by the 18 g group. This may indicate that tooth movement is not force dependent and supports the conclusion of Pilon *et al.* (1996) that the magnitude of force is not decisive in determining the rate of bodily tooth movement, but that individual characteristics are. The obvious difference between the 8 and 18 g groups was the significant difference in CAV and CAPL on day 7. It is suggested that a continuous 18 g force with super elasticity delays PDL reconstruction, implying that the severely compressed area might extend peripherally from the primary compressed region, as reported by Satoh *et al.* (1995).

As shown in Figure 5, PDL expansion was noted in the 8 and 18 g groups on day 28, and the CAPL of these two groups were significantly larger than that of the controls. In contrast, evident expansion was not seen in other experimental groups on the same day, and there was no significant difference between these groups and the controls. Due to widening of the PDL with undermining bone resorption on the compressed side, further tooth movement might occur beyond the normal PDL width after 28 days. A previous study (Noda *et al.*, 2000) found that degenerating

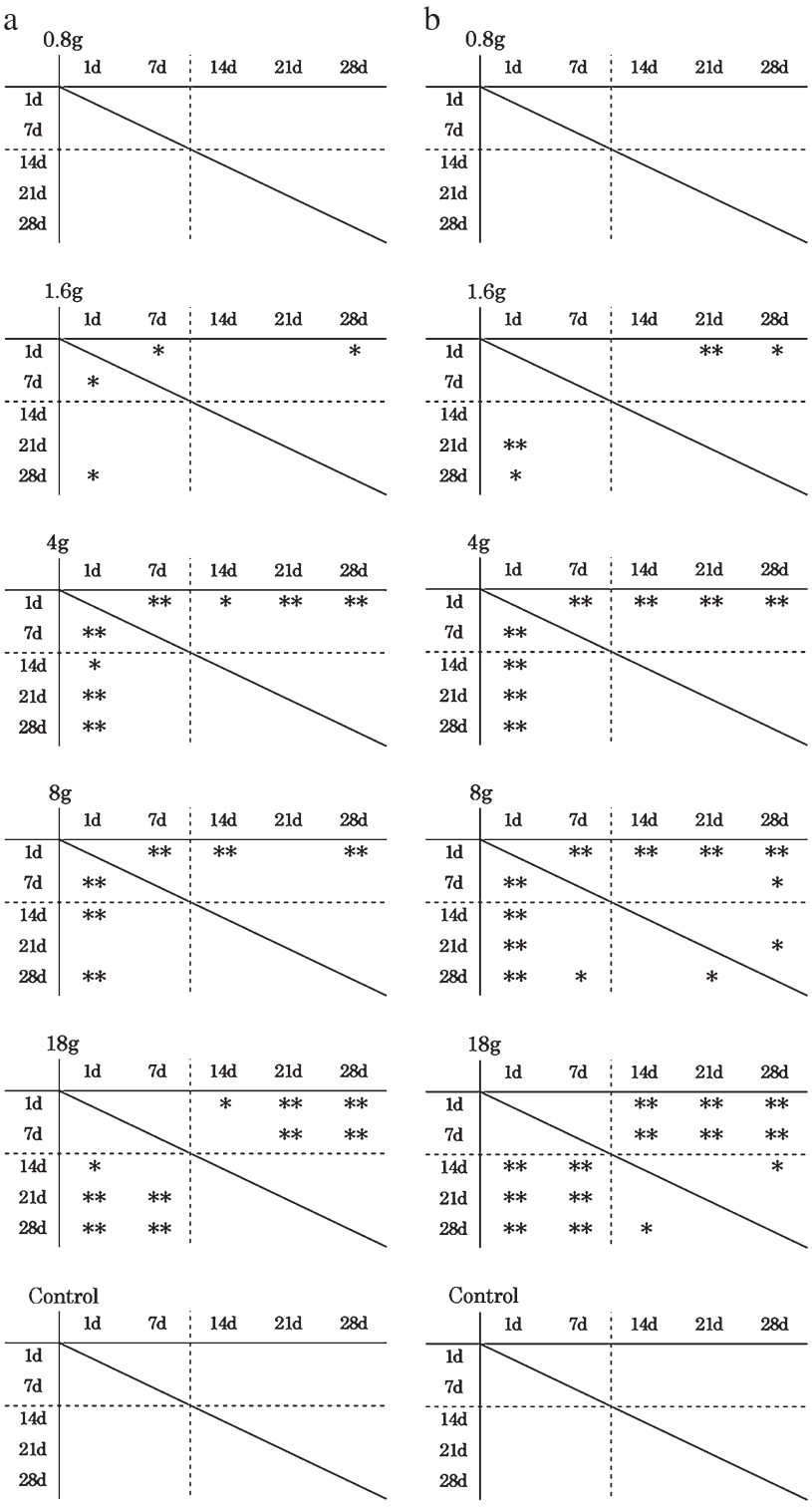

Figure 7 Tukey's HSD test on the time course changes of total cross-sectional areas of blood vessels (a) and the periodontal ligament without blood vessels (b) in the experimental and control groups an (1, 7, 14, 21, and 28 days after tooth movement).*$P < 0.05$, **$P < 0.01$.

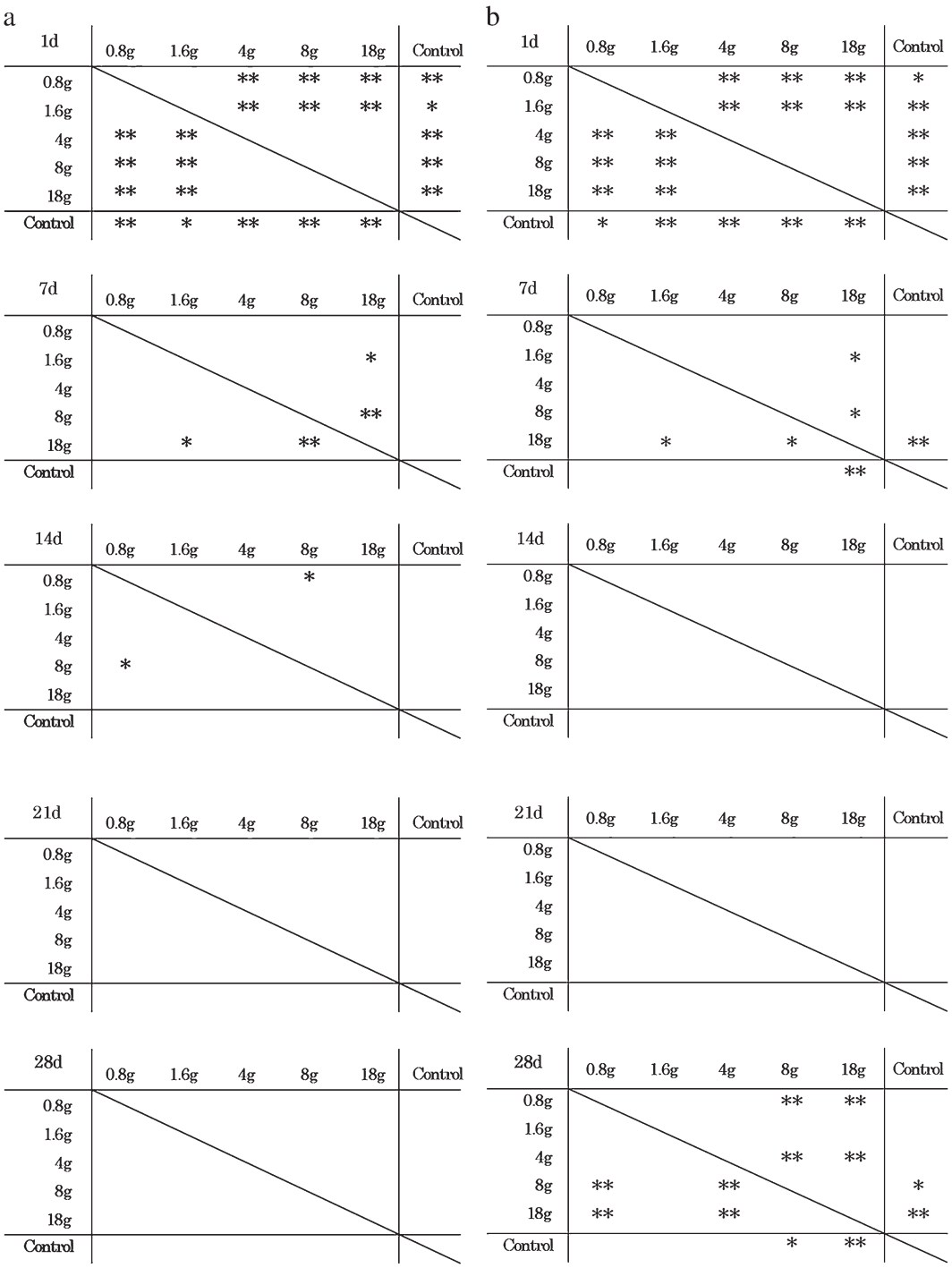

Figure 8 Tukey's HSD test of the total cross-sectional areas of blood vessels (a) and periodontal ligament without blood vessels (b) during the experimental periods. $*P < 0.05$, $**P < 0.01$.

tissue in 8 and 18 g groups was significantly larger than that in a 4 g group, while tooth movement in these three groups was comparable and longer than that in 1.6 and 0.8 g groups (Figure 4). From the above-mentioned results, it can be assumed that a force magnitude that results in degeneration of tissue and undermining bone resorption might be required to induce efficient tooth movement.

Conclusions

The findings of the present study of tooth movement of the upper first molars of Wistar rats were as follows:

1. Vascular structures were seen in the compressed PDL of the experimental groups when a force magnitude of less than 1.6 g was used.

2. CAV and CAPL in the experimental groups following an application of a force over 4 g were significantly smaller than in the 0.8, 1.6 g, and control groups.
3. Significant expansion of blood vessels was seen in the 1.6 to 8 g experimental groups on day 1 to 7 after tooth movement.
4. Significant expansion of the PDL was seen in the experimental groups over 8 g on day 28 after tooth movement.

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