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Time-lapse observation of rat periodontal ligament during function and tooth movement, using microcomputed tomography

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SUMMARY The aim of this study was to observe the time-lapse changes in the rat periodontal ligament (PDL) during function and tooth movement. Under Nembutal anaesthesia, time-lapse changes in the thickness of the PDL of the first molars were investigated in five 12-week-old adolescent rats with microcomputed tomography. Three-dimensional (3D) images were reconstructed from the data. Histological observation was also performed, using undecalcified frozen sections of the maxillary first molar area.

The PDL appeared as a radiolucent furrow on the 3D images. A slight change in the thickness of the PDL was observed 1 hour after initiation of orthodontic force loading, which became significant after 6 hours, with the appearance of pressure–tension zones during the tooth movement. These changes were more significant 3 days after orthodontic loading.

Histological observation of the lingual cervical PDL (pressure zone) in nine 12- to 13-week-old rats demonstrated that the periodontal space had become narrow and the cellular elements appeared to be densely packed in the narrowed PDL 6 hours after orthodontic loading. Degeneration of tissues appeared 3 days after loading. Observation of the buccal cervical PDL (tension zone) demonstrated that the PDL was extended 6 hours after orthodontic force loading, and the extension continued for up to 3 days. Alkaline phosphatase activity was distributed in the PDL, except for the degenerating tissues in the pressure zone 3 days after loading.

The results suggest that the periodontal reaction was initiated within 6 hours after orthodontic force loading, which was related to the structural changes of the PDL. The changes probably induced an early response in individual cells of the PDL.

Introduction

In orthodontics it is important to investigate the change in thickness of the periodontal ligament (PDL) during tooth movement as a change in thickness of the PDL influences the intra- and extracellular environments of the PDL cells, such as reorganization of cytoskeleton and their surrounding tissue pressure (Masella and Meister, 2006), which induce different gene expression and functions of the cells, and consequently histological tissue reactions occur in the PDL. The initial change in thickness of the PDL is the critical trigger for biological reaction of the PDL during tooth movement.

However, there is a lack of information regarding when the initial change in thickness occurs and it is difficult to identify the initial change in thickness in the PDL during tooth movement. The PDL has a viscoelastic property and does not compress rapidly in response to orthodontic force, which makes it difficult to identify the precise timing of the change in thickness of the PDL.

Much attention has been given to elucidate the initial changes of the PDL during tooth movement. In a histological study, the initial change in thickness of PDL could not be identified due to technical difficulties. The thickness of the PDL is easily influenced by tissue preparation, such as handling, decalcification, and dehydration of the specimen. Thus, some studies (Reitan and Kvam, 1971; Noda *et al.*, 2000) have shown the histological tissue changes of the PDL several days after tooth movement.

Examination of the changes in the thickness of PDL make it possible to identify the exact timing of the cellular reaction at gene expression level, which is a requisite for understanding the biological process of tooth movement.

In this context, the changes in thickness of the PDL of maxillary first molar were investigated in the functional state during tooth movement, with microcomputed tomography (μ-CT). In addition, histological evaluation of the PDL was performed in the initial stages of tooth movement.

Materials and methods

Observation with μ-CT

Five adolescent (12-week-old) rats were used in this study. All procedures described below followed the guidelines and regulations of Tsurumi University for Animal Research.

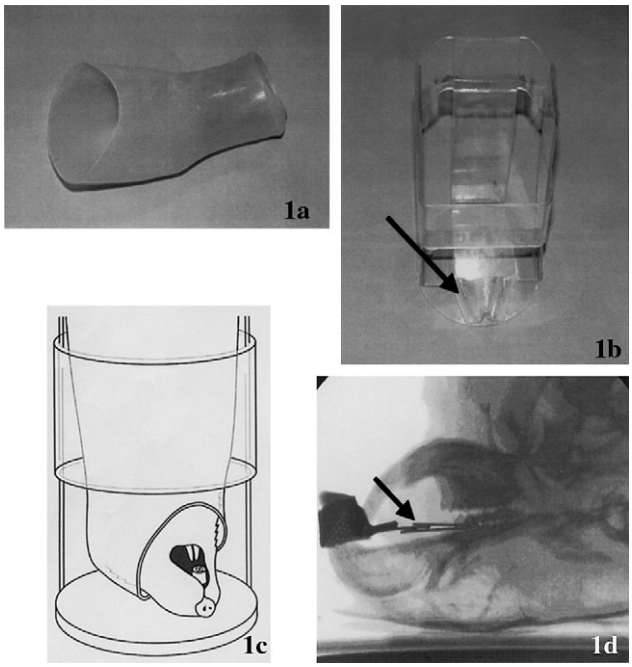

Figure 1 Device to fix each rat on the specimen stage of the microcomputed tomographic apparatus and the orthodontic fixed appliance. (a) A polystyrene cylindrical tube. (b) A plastic case to fix the polystyrene cylindrical tube. A half-conical tube plate (arrow) was placed on the base plate to fix the head of a rat. (c) A schematic drawing of a rat in the tube. (d) The fixed appliance used in this study. Helical loop springs (arrow) were used to move the maxillary first molars lingually. The occlusal plane of the maxillary molars was almost parallel to the specimen stage.

Prior to observation with μ-CT, a device was constructed to fix each rat on the specimen stage of the μ-CT. The device consisted of two elements: a polystyrene tube (Figure 1a) to accommodate the body and a plastic case to fix the tube (Figure 1b,c). The case, made of thermoplastic material, was composed of a round base plate, lateral and back plates, circumferential plates (front and back) adhered to each other to fix the polystyrene tube, and a half-conical tube plate attached to the base plate to fix the head of the rat.

Under anaesthesia with Nembutal, an orthodontic appliance was fixed on the maxillary incisors (Figure 1d). The rat was placed in the device and the head in the half-conical tube plate with the occlusal plane of the maxillary molars almost parallel to the round base plate (Figure 1d). Then, the device with the rat was set on the specimen stage of the μ-CT with double-sided adhesive tape. The μ-CT images were taken at a tube voltage of 70 kV, tube current of 100 μm, and isotropic voxel dimension of 13 μm (MCT-CB 100MF, Hitachi Medico Co., Tokyo, Japan) without tooth movement (control). Lingual movement of the maxillary first molars was then initiated with an orthodontic force of 10 g. The μ-CT images were also obtained at 1 hour, 6 hours, and 3 days after initiation of orthodontic force loading.

The obtained μ-CT data were transferred to a personal computer, and three-dimensional (3D) images of the bone and roots were reconstructed using image processing software (tri-CT bon, Ratock, Tokyo, Japan). The thickness of the cervical PDL showing maximal compression at the distolingual root and maximal expansion at the distobuccal root was measured.

Histological observation

Nine adolescent rats (12–13 weeks old) were used in this part of the study. The rats were divided into three groups: a control (without tooth movement), and a 6 hour, and 3 day group.

Under Nembutal anaesthesia, the rats were killed and the maxillae were excised out and rapidly immersed in isopentane cooled with liquid nitrogen. The frozen tissues were trimmed into smaller blocks containing the first molar with a dental diamond disk in the frozen condition. The frozen tissues were then embedded in pre-cooled optimal cutting temperature (OCT) compound (Miles Inc., Torrance, California, USA) and returned to liquid nitrogen until the OCT compound was completely frozen. The frozen blocks were stored in a refrigerator at −80°C. They were then mounted on stubs precooled at −25°C in a cryostat (Reitz, Nussloch, Germany) with OCT compound and frontally sectioned using a super-hard tungsten steel knife (Meiwa Shoji Ltd., Tokyo, Japan; Nakamura *et al.*, 1994). Serial frontal sections, 5 μm thick, were collected individually with adhesive tape (Kawamoto and Shimizu, 1986) and were then freeze dried for 1 hour in the cryostat. The tape with the attached section was transferred to a glass slide. Some sections were fixed with buffered formalin for 60 seconds, washed carefully with distilled water, stained with 0.5 per cent toluidine blue for 1 minute at room temperature, and mounted under a coverslip with glycerin.

After histological observation of the toluidine blue-stained sections, the consecutive sections were prefixed with 100 per cent ethanol for 60 seconds and rinsed with distilled water. They were then incubated in a medium which detects alkaline phosphatase activity (ALP activity: azo dye method; Mayahara *et al.*, 1969) for 90 seconds at room temperature. After incubation, the sections were post-fixed with buffered formalin.

Results

Observation with μ-CT

The PDL of the maxillary first molar of the rat could be observed during function using the device to fix the head of the rat. The PDL of the maxillary first molar without tooth movement (control) was interposed between the bone and roots, covering the roots (Figure 2a).

The 3D reconstructed images of the first molar area revealed the relationships of the roots and the surrounding bone (Figure 2b). The PDL was observed as a radiolucent band or channel. The thickness of the PDL was almost constant, and all the five roots were covered with PDL (Figure 2c–f). The average thickness of the PDL at the

cervical area was 0.09 ± 0.007 mm on the lingual side and 0.09 ± 0.010 mm on the buccal side (Table 1).

One hour after orthodontic force loading, a slight visible change had occurred in the thickness of the PDL. The lingual PDL was slightly thinner than the buccal PDL (Figure 3a,b). Frontal 3D images of the mesiolingual, mesiobuccal, distolingual, and distobuccal roots showed that the thickness of the lingual PDL differed from that of the buccal PDL (Figure 3c–f).

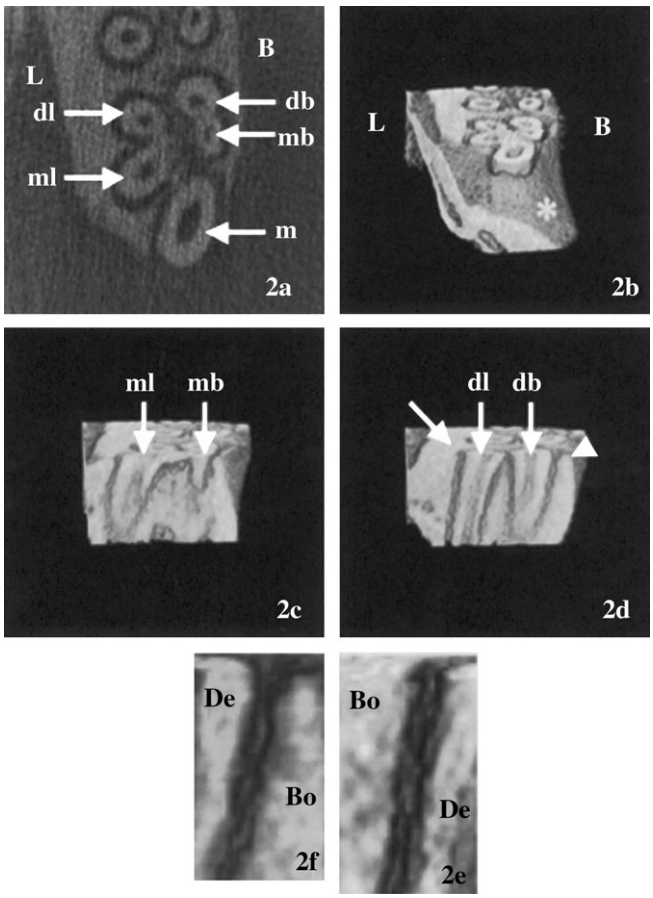

Figure 2 Microcomputed tomographic and three-dimensional reconstructed images. The periodontal ligament (PDL) of a maxillary first molar without tooth movement (control) appeared as a concentric circle, covering the roots (a and b). The thickness of the PDL was almost uniform for all roots root (c–f). (e) and (f) are enlarged views of Figure 1d. The PDL was observed as a radiolucent furrow. Frontal images cut at the mesiolingual and mesiobuccal roots (c) and at the distolingual and distobuccal roots (d). There are five roots in the rat maxillary first molar. m, mesial root; mb, mesiobuccal root; ml, mesiolingual root; db, distobuccal root; dl, distolingual root, Bo, bone; De, dentine; B, buccal side; L, lingual side.

Six hours after orthodontic force loading, the PDL exhibited tension and pressure zones (Figure 4a,b), characteristic features of tooth movement. Frontal sections of the 3D image at the mesiolingual, mesiobuccal (Figure 4c), distolingual, and distobuccal (Figure 4d) roots demonstrated that the lingual PDL had become thinner (Figure 4e) and the buccal thicker (Figure 4f). A pressure gradient, with the highest pressure at the cervical area of the PDL on the lingual side, was observed, suggesting that the maxillary first molar had tipped lingually. The radiolucent furrow at the cervical region of the lingual PDL appeared somewhat radiopaque (Figure 4e). The average thickness of the PDL significantly decreased to 0.058 ± 0.004 mm in the

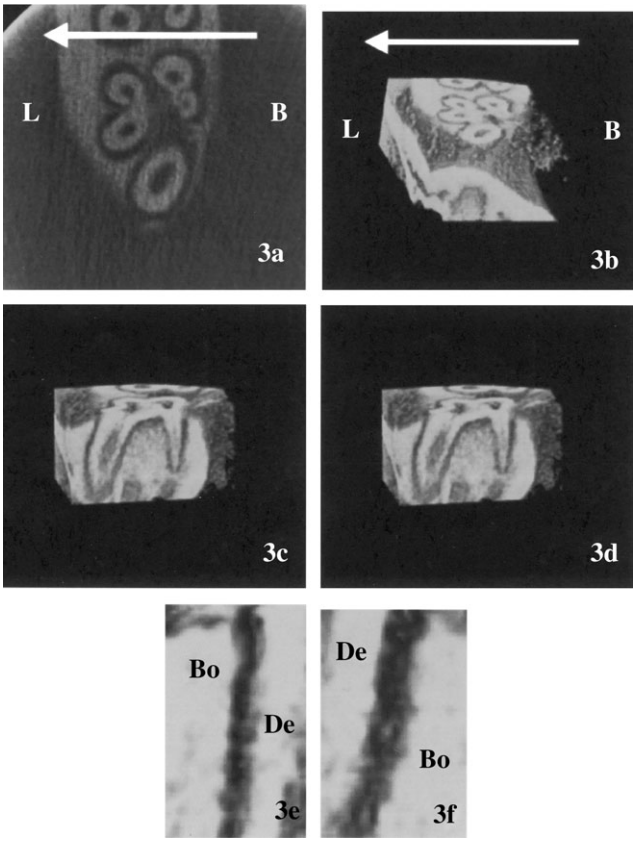

Figure 3 Microcomputer tomographic and three-dimensional reconstructed images 1 hour after orthodontic loading. The lingual periodontal ligament (PDL) was slightly thinner than the buccal (a and b). At the mesiolingual and mesiobuccal, and distolingual and distobuccal roots, a slight change in the thickness of the PDL has occurred (c and d). (e) and (f) are enlarged views of (d). Bo, bone; De, dentine; L, lingual side; B, buccal side; large arrow, direction of orthodontic force.

Table 1 Measurement of thickness of the periodontal ligament.

	Lingual side (mm)	*P* value	*P* value	Buccal side (mm)	*P* value	*P* value	*P* value
Control	0.090 ± 0.007	—	—	0.090 ± 0.010	—	—	1
6 hours	0.058 ± 0.004	0.034	—	0.128 ± 0.013	0.041	—	—
3 days	0.038 ± 0.004	0.034	0.025	0.158 ± 0.008	0.041	0.039	—

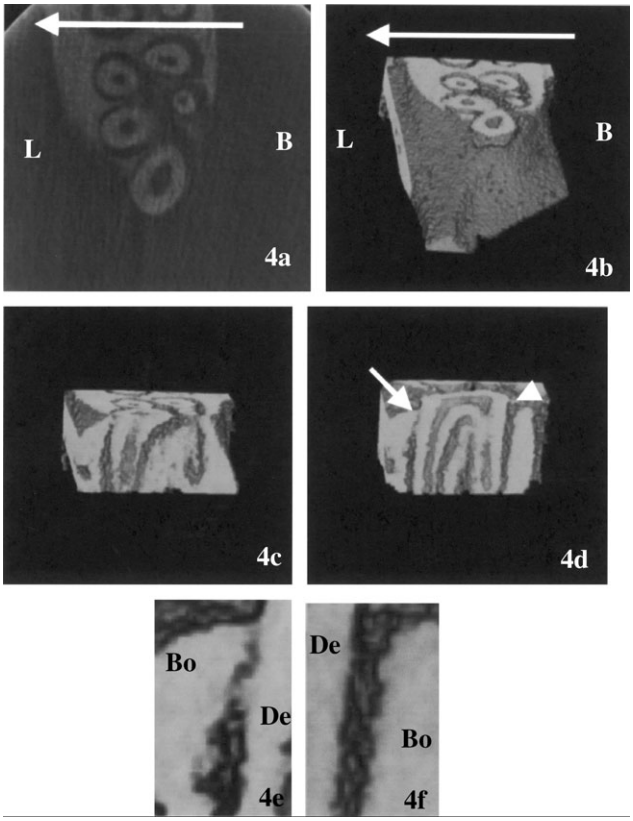

Figure 4 Microcomputed tomographic and three-dimensional (3D) reconstructed images 6 hours after orthodontic loading. The lingual periodontal ligament (PDL) was thinner and the buccal PDL was thicker (a and b), indicating the appearance of pressure–tension zones. Frontal 3D images demonstrated that the maxillary first molar had tipped lingually (c–f). (e) and (f) are enlarged views of (d). The radiolucent furrow at the cervical region of the lingual PDL showed some radiopacity (e). L, lingual side; B, buccal side; Bo, bone; De, dentine; large arrow, direction of the orthodontic force.

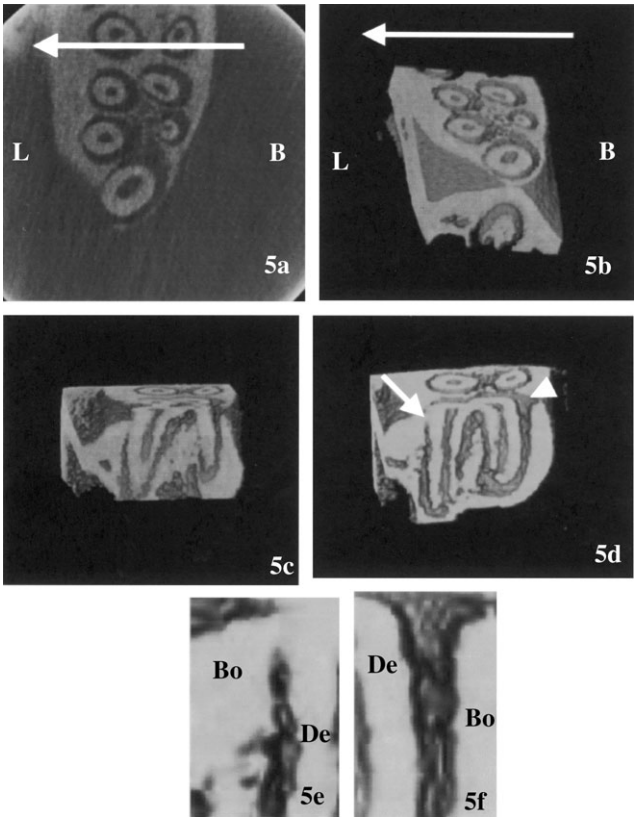

Figure 5 Microcomputed tomographic and three-dimensional reconstructed images 3 days after orthodontic loading. The lingual periodontal ligament (PDL) remained thinner and the buccal PDL thicker (a and b). Frontal three-dimensional images demonstrated that the maxillary first molar tipped further lingually (c–f). The PDL at the cervical area was thinner and showed radiopacity similar to that of calcified bone and dentine. (e) and (f) are enlarged views of (d). L, lingual side; B, buccal side; Bo, bone; De, dentine; large arrow, direction of the orthodontic force.

pressure zone and significantly increased to 0.128 ± 0.013 mm in the tension zone (Table 1).

Three days after orthodontic force loading, the changes in thickness of the PDL were more apparent in the pressure and tension zones (Figure 5a,b), as demonstrated by the frontal 3D images. Both the lingual roots were very close to the alveolar bone at the cervical area (Figure 5c–e) and radiopacity increased and was similar to that of calcified bone and dentine (Figure 5e). The average thickness of the PDL decreased further to 0.038 ± 0.004 mm in the pressure zone and increased further to 0.158 ± 0.008 mm in the tension zone (Table 1).

Histological observation

Undecalcified sections of the first molar area in the control group revealed that the PDL was interposed between the root and alveolar bone. Osteoblasts and cementoblasts were seen on the surfaces of the bone and cementum, respectively. The fibroblasts were scattered in the PDL (Figure 6a).

In contrast to the observations in the control group, structural changes occurred in the PDL 6 hours after orthodontic force loading (Figure 7a,b). In the pressure zone (lingual cervical PDL), the periodontal space had become narrower, the cellular elements appeared to be densely packed in the narrowed PDL, and the typical arrangement of the periodontal fibres was not apparent (Figure 7a). However, there was no sign of degeneration of the tissues. In the tension zone (buccal cervical PDL), the periodontal space had become wider and the periodontal fibres appeared stretched between the bone and root (Figure 7b).

Three days after orthodontic force loading, the cellular elements were not detectable, indicating that tissue degeneration had occurred in the PDL in the pressure zone (Figure 8a). In the tension zone, the expansion of periodontal space was more evident between the bone and root (Figure 8b).

ALP activity was distributed not only on the bone surface but also throughout the PDL in the sections of

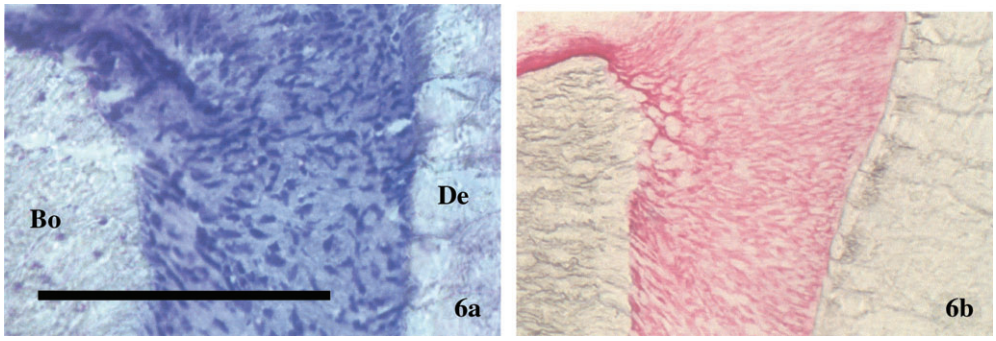

Figure 6 Cervical periodontal ligament (PDL) of a maxillary first molar of the control rat. Osteoblasts and cementoblasts were present on the surfaces of the bone and cementum, respectively, and fibroblasts were scattered in the PDL (a). Alkaline phosphate (ALP) activity was distributed not only on the bone surface but also throughout the PDL (b). Bo, bone; De, dentine. (a) toluidine blue stain, bar: 100 µm, ×200 and (b) ALP activity, ×200.

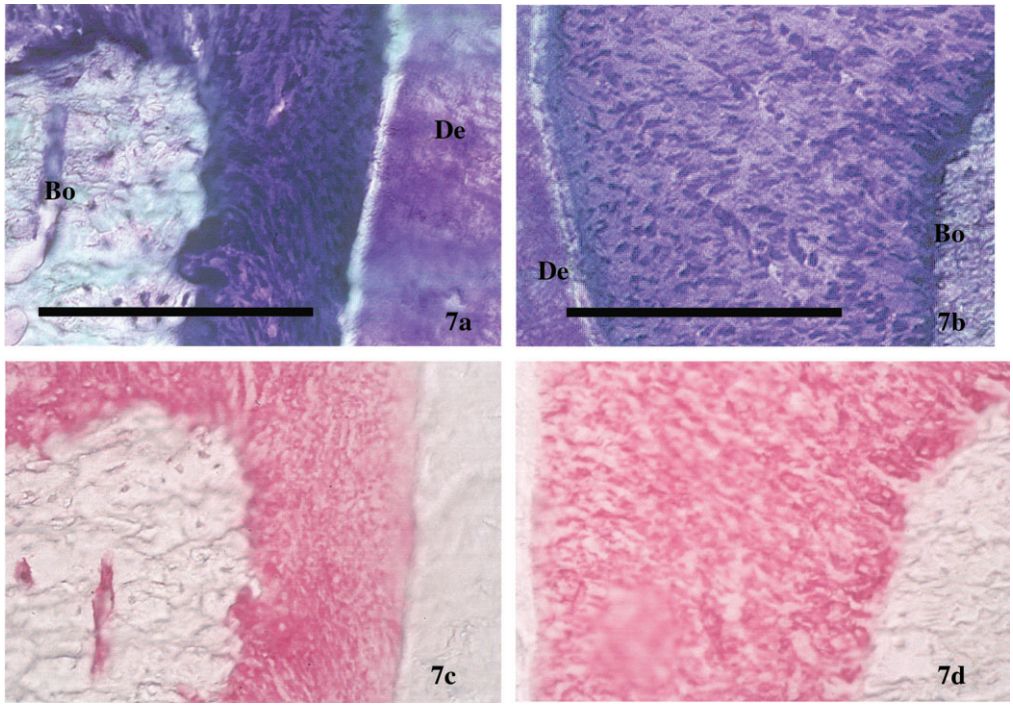

Figure 7 Cervical periodontal ligament (PDL) 6 hours after orthodontic force loading. In the lingual PDL (pressure zone), the periodontal space became narrow, the cellular elements appeared to be densely packed in the narrowed PDL, and the typical arrangement of the periodontal fibres was not apparent in the PDL (a). In the buccal PDL (tension zone), the periodontal space became wide and periodontal fibres were stretched between the bone and root (b). Alkaline phosphatase (ALP) activity was distributed in both pressure and tension zone of the PDL (c and d). Bo, bone; De, dentine. (a) and (b) toluidine blue stain, bar: 100 µm, ×200 and (c) and (d) ALP activity, ×200.

the control group (Figure 6b). ALP activity was observed in both pressure and tension zones of the PDL, 6 hours after orthodontic force loading. It was located along the bone surface and throughout the PDL (Figure 7c,d). Three days after orthodontic force loading, however, no activity was observed in the degenerating tissues in the pressure zone (Figure 8c). In the tension zone, activity was observed along the bone surface and also in the PDL (Figure 8d).

Discussion

Micro-CT is useful for observation of the microarchitecture of bone specimens and 3D images make it easier to comprehend the complicated structures (Kobayashi *et al.*, 2003; Yang *et al.*, 2003; Jiang *et al.*, 2005; van Ruijiven *et al.*, 2005). No other study appears to have elucidated the changes in the specimen from hour to hour. In orthodontic tooth movement, the thickness of the PDL varies hourly. It is important to examine the temporal changes in the

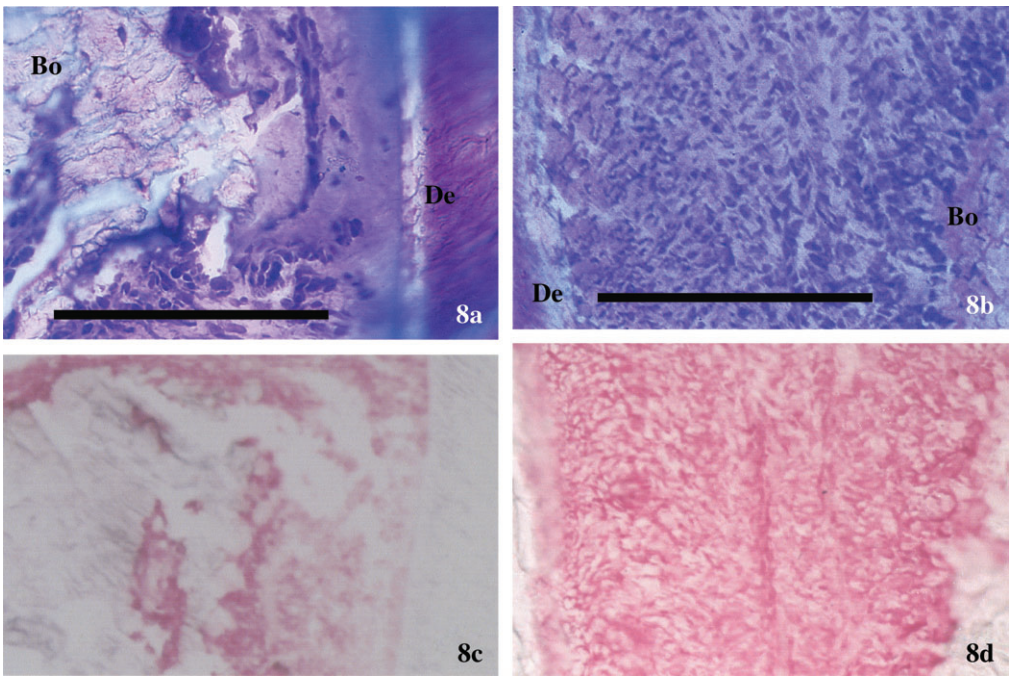

Figure 8 Cervical periodontal ligament (PDL) 3 days after orthodontic force loading. In the lingual PDL (pressure zone), the cellular element appeared to have been lost (a), and in the buccal PDL (tension zone) the expansion of periodontal space was more evident between the bone and root. Alkaline phosphatase (ALP) activity was not detected in the degenerating tissues in the pressure zone (c) and was distributed on the bone surface and in the PDL in the tension zone (d). Bo, bone; De, dentine. (a) and (b) toluidine blue stain, bar: 100 μm, ×200 and (c) and (d) ALP activity, ×200.

thickness of the PDL during the initial stages of tooth movement because these changes cause alterations in the gene expression in individual cells of the PDL.

In this research, the time-lapse changes of rat PDL during function and tooth movement were examined. The fixation device for the μ-CT made it possible to observe the PDL and to obtain the time-lapse changes of the PDL during tooth movement. With this method, the PDL can be observed whenever required, by further anaesthetizing the rats. The method used can also be applied for observation of time-lapse changes in the dentomaxillofacial morphology of small animals.

The PDL is conventionally observed as a radiolucent band between the alveolar bone and tooth. In the 3D images, the PDL appeared as a radiolucent furrow interposed between bone and tooth. The 3D images of the PDL provide an appearance of depth, unlike those of flat bone and tooth.

In the present study, the initial changes in thickness of the PDL were revealed. A sign of change in thickness of the PDL was observed 1 hour after initiation of orthodontic force loading and the evident changes were noted 6 hours after orthodontic force loading. Therefore, the early response of the PDL occurred within 6 hours after initiation of orthodontic force loading. These findings are also supported by the histological observation. The appearance of the PDL in the pressure and tension zones was structurally different from that of the control, although the distribution of ALP activity was similar. In the pressure zone, the cellular elements appeared to be densely packed in the narrowed PDL and the typical arrangement of the periodontal fibres was not apparent. In the tension zone, the periodontal fibres appeared to be stretched between the bone and roots. These environmental changes around the cells in the initial stage of tooth movement induce alteration of the gene expression in individual cells of the PDL, and consequently cause an inflammatory reaction and bone resorption (Nakamura *et al.*, 1996, 2003) in the pressure zone, and bone formation in the tension zone. Further studies will be necessary to investigate the initial cellular reaction at gene expression level.

The change in thickness 6 hours after orthodontic force loading was also accompanied by an increase in radiopacity in the pressure zone of the PDL, which is related to the histological observations that the cellular elements were densely packed in the narrowed PDL. The radiopacity further increased in the lingual cervical PDL and became similar to that of calcified tissues 3 days after treatment. This could be due to the presence of ectopic calcification in the degenerating tissues during tooth movement (Nakamura *et al.*, 1996), which prevents direct contact between tooth and bone (Nakamura *et al.*, 2003). Histological observation in this investigation revealed the presence of degenerating tissues in the pressure zone of the PDL. ALP activity was not found in the degenerating tissues (Oikawa *et al.*, 2004),

suggesting that qualitative changes at tissue level had already occurred in the PDL.

Conclusion

Periodontal reactions were initiated within 6 hours after orthodontic force loading, which was related to the structural changes of the PDL.

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