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Restoration of mechanical strength and morphological features of the periodontal ligament following orthodontic retention in the rat mandibular first molar

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SUMMARY Biomechanical properties and morphological features of the periodontal ligament (PDL) in the rat mandibular molars were examined during orthodontic retention. Seventy-three male rats of the Wistar strain, 8 weeks of age, were used for biomechanical analysis and six rats for morphological analysis. An elastic band was inserted between the mandibular first and second molars for 4 days; after removal of the elastic band the interdental space was filled with resin for 4 and 8 days.

The maximum shear stress, tangent modulus, and failure strain energy density of the PDL of the first molar in the experimental animals decreased markedly following application of an orthodontic force. They increased rapidly and were restored completely to the control levels by the 8th day after retention. Light microscopy showed severe compression and extension of the PDL in the experimental animals on the 8th day after retention. Birefringent collagen fibre bundles running across the compressed and expanded PDL were observed, although they appeared to be thinner with less insertions into the alveolar bone or cementum in the experimental animals than in the controls. This suggests that the periodontal collagen fibres were partially reorganized and rearranged during retention. The reorganization and rearrangement of periodontal collagen fibres seemed to be partly related to the restoration of mechanical strength of the rat molar PDL during the 8 days of retention.

Introduction

It has been shown that the maximum shear load of the periodontal ligament (PDL), estimated by extracting a tooth from its socket in the dissected jaw, decreases rapidly and markedly after application of an orthodontic force in the rat mandibular or maxillary molars (Ohkawa, 1982; Tsuruta *et al*., 1982; Hong, 1990; Hong *et al*., 1992). Restoration of the maximum shear load of the ligament occurs rapidly in accordance with the closure of the interdental space after removal of the orthodontic force (Tsuruta *et al*., 1982). Restoration of the maximum shear load of the PDL also occurs after orthodontic retention performed by filling the interdental space with light-cured resin (Hong *et al*., 1992).

Additional information concerning the biomechanical properties of connective tissues can be obtained when the stress–strain curve is recorded rather than measuring the maximum shear load from the load–deformation curve (Viidik, 1980; Mandel *et al*., 1986; Komatsu, 1988). Analysis of stress–strain curves obtained from the PDL of the rat mandibular first molar has been made after application of an orthodontic force (Fukui, 1993). However, the stress–strain behaviour with regard to the structure of the periodontal collagen fibres has not been examined in the ligament of the rat molar after orthodontic retention, although there have been several reports on the histological changes of the PDL incident to orthodontic retention (Reitan, 1959, 1967, 1969; Tensin *et al*., 1990; Kusters *et al*., 1991; Reitan and Rygh, 1994).

The aims of the present study were to analyse stress–strain curves obtained from the PDL after orthodontic retention and to determine whether the mechanical behaviour of the ligament is related to structural changes of the collagen fibres observed by a polarized light microscope in the rat mandibular first molar.

Materials and methods

Seventy-three male rats of the Wistar strain, 8 weeks of age, with an average body weight of 210 ± 12 (SD) g, were used for biomechanical analysis. They were divided into three experimental and four control sub-groups of 10 or 11 animals. All animals were fed on a powdered diet and given water *ad libitum* during the experimental period and for about 3 weeks beforehand. In the three experimental sub-groups, a latex elastic band (No. 404-126; Unitek, Monrovia, USA) with an average thickness of 616 ± 45 (SD) μm was inserted into the interproximal space between the right mandibular first and second molars while the animals were under ether anaesthesia (Fukui, 1993) at the beginning of the experimental period (day 0). In the four control sub-groups, an elastic band was not inserted between the teeth, but the rats were also anaesthetized with ether and formed the control group. On day 0, the rats in one control sub-group (0-day control sub-group) were killed by decapitation under ether anaesthesia. On the 4th day after insertion of the elastic band, the rats in one experimental sub-group (4-day experimental sub-group) and one control sub-group (4-day control sub-group) were killed. On the same day, in the remaining two experimental sub-groups, the elastic band was removed and orthodontic retention was performed by filling the interdental space with light-cured resin (Transbond, Unitek/3M) (Hong *et al*., 1992). The rats of two experimental sub-groups were killed on the 8th (8-day experimental sub-group) and 12th (12-day experimental sub-group) days of the experimental period, respectively. The rats in the two control sub-groups (8- and 12-day control sub-groups) were also killed on the 8th and 12th day of the experimental period as the experimental sub-groups. The data from two rats in the 8-day control sub-group, two rats in the 12-day control sub-group, and one rat in the 12-day experimental sub-group were excluded, because they died by anaesthetic accident. Experimental protocols concerning animal handling were reviewed and approved by the Institutional Animal Care Committee of Tsurumi University School of Dental Medicine.

Immediately after death, the right mandibles were dissected and adherent soft tissues removed. A transverse section [462 ± 65 (SD) μm thickness] of the mandibular first molar with its surrounding PDL and alveolar bone was cut through an axis perpendicular to the long axis of the mesial root at the middle part of the root (Komatsu, 1988; Komatsu and Chiba, 1993), using a saline-cooled, low-speed bone saw (Isomet, Buehler, IL, USA).

Radiographs of the transverse sections were taken in a soft X-ray apparatus (Type EMB; Softex, Tokyo, Japan). The radiographic images were received by a television camera (CTC-2600; Ikegami Tsushinki, Tokyo, Japan) linked to an image analyzer (Luzex-3U; Nikon, Tokyo, Japan). From the recorded data, the perimeters of the cementum and socket wall, and the sectional area of the PDL in the mesial root of the mandibular first molar were measured. The area of the PDL facing the cementum (thickness of section × perimeter of cementum) and the average width of the PDL [sectional area of the PDL/(perimeter of cementum + perimeter of socket wall)/2] were calculated (Komatsu, 1988; Komatsu and Chiba, 1993).

A transverse section of the mesial root of the mandibular first molar was pushed out of its surrounding alveolar bone using a mechanical testing machine (S-100; Shimadzu, Kyoto, Japan) as described previously (Komatsu, 1988; Chiba *et al*., 1990). The root was loaded at 5.0 mm/minute in an extrusive direction until failure of the PDL. The data were incorporated into a computer (PC9801VM; NEC, Tokyo, Japan). For each specimen, a load–deformation curve was reconstructed from the data stored in the computer and then transformed into a stress–strain curve (Komatsu and Chiba, 1993). From the stress–strain curve, maximum shear stress (MPa), maximum shear strain, tangent modulus (MPa), and failure strain energy density (MJm^{-3}) were estimated (Mandel *et al*., 1986; Komatsu, 1988; Komatsu and Chiba, 1993). The data from two rats in the 8-day control sub-group and one rat in the 12-day control sub-group were excluded, because the surrounding alveolar bone was broken during mechanical testing. The time between killing of the animals and mechanical testing ranged from 168 to 236 minutes during which time the specimens were kept in cold saline.

To measure the interdental space, the 12-day experimental sub-group was used and hydrophilic rubber impressions (Exaflex; GC, Tokyo, Japan) of the right mandibular dental arches were taken under ether anaesthesia on days 0 (before insertion of an elastic band), 1, 2, 4, 6, 8, 10, and 12. From the rubber impressions, plaster models of the dental arch were reconstructed. For each plaster model, the distance between the mesial end of the first molar and the distal end of the third molar along the midline of the dental arch (Figure 1) was measured with a profile projector (V-16D; Nikon, Tokyo, Japan). The difference in the lengths of the dental arches before and after insertion of an elastic band was regarded as the interdental space.

For histological analysis, another six rats were used. They were divided into two equal groups and treated as the 12-day control and experimental sub-groups, respectively. At the end of the experimental period, the

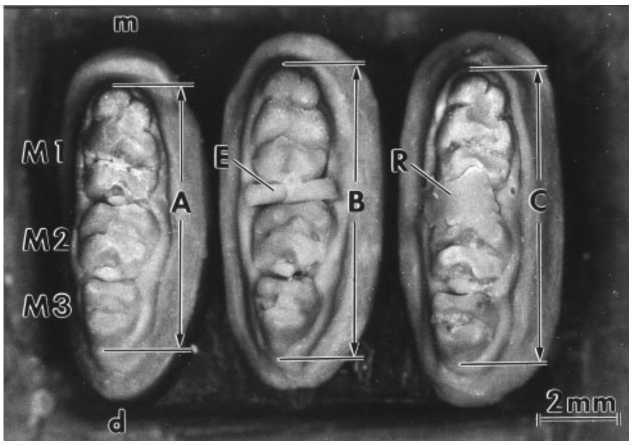

Figure 1 Plaster models of the dental arches obtained from a rat before (left) and after (middle) insertion of an elastic band (E) between the first (M1) and second (M2) molars, and after retention (right) with light-cured resin (R). A, B, and C represent the lengths of the dental arches. The differences in length between B and A, and between C and A were regarded as the interdental spaces. m, mesial side; d, distal side; M3, the third molar.

rats were perfused with 10 per cent neutral buffered formalin for 10 minutes under ether anaesthesia. Their right mandibles were dissected, fixed and decalcified in Bouin fixative solution, and embedded in gelatin. Midsagittal sections were cut at a microtome (Model 1320; Leitz, Wetzler, Germany) setting of 26 μm, stained with haematoxylin and eosin, mounted in glycerin jelly, and observed under light and polarized light microscopes.

The differences in the mean values between the experimental and respective control sub-groups were compared by *t*-test. When indicated, one-way analysis of variance (ANOVA) was used to examine differences between mean values among the control or experimental sub-groups.

Results

Weights of animals and mandibles

Table 1 shows weights of animals and mandibles during the experimental period. The average weights of animals ranged from 212 to 237 g in the control and 214 to 234 g in the experimental sub-groups. The average weights of mandibles ranged from 336 to 365 mg in the control and from 346 to 366 mg in the experimental sub-groups. There were no significant differences in either the weights of animals or mandibles between the experimental and respective control sub-groups.

Table 1 Weights of animals and right mandibles.

Days	Weight of animals (g)			
	Experimental sub-groups		Control sub-groups	
	Mean ± SD	*n*	Mean ± SD	*n*
0	–	–	212 ± 13	10
4	214 ± 10	10	218 ± 11	11
8	225 ± 15	10	227 ± 9	7
12	234 ± 16	9	237 ± 19	8
	Weight of right mandibles (mg)			
0	–	–	336 ± 13	10
4	346 ± 10	10	340 ± 11	11
8	365 ± 15	10	357 ± 20	7
12	366 ± 18	9	365 ± 13	8

On day 0, an elastic band was inserted between the mandibular first and second molars in all experimental sub-groups. On day 4, the band was removed and the interdental space was filled with resin in the 8- and 12-day experimental sub-groups. Mean ± 1 SD is shown for each sub-group. There were no significant differences in either the weight of the animals or the right mandibles between the experimental and respective control sub-groups. At the beginning of the experiment for studying mechanical properties, each group consisted of 10 or 11 rats (total 73 rats). However, data from two rats in the 8-day control sub-group, two in the 12-day control sub-group and one in the 12-day experimental sub-group were excluded, because they died accidentally under anaesthesia. Data from two rats in the 8-day control sub-group and one rat in the 12-day control sub-group were also excluded, as the surrounding alveolar bone was broken during mechanical testing.

Interdental space

Figure 2 shows the changes in the interdental space between the mandibular first and second molars obtained from the 12-day experimental sub-group during the experimental period. An elastic band was inserted between the teeth on day 0, and the interdental space was filled with resin after removal of the band on day 4. The interdental space increased markedly and immediately after insertion of the band (the rate being 0.32 mm/day for the initial 24 hours) and increased at a slow rate of 0.05 mm/day during the following 3 days before retention. After retention, the average interdental spaces were relatively constant, ranging from 0.42 to 0.53 mm during the retention period of 8 days.

Stress–strain curves

The shear stress–strain curves obtained from the PDL of the mesial root of the rat mandibular first molar in the control and experimental sub-groups are shown in Figure 3. Only the rising parts of the curves are shown so that the end point with vertical and horizontal bars in each curve represents the mean ± 1 SD for maximum shear stress and maximum shear strain, respectively. The shapes of the stress–strain curves and stress levels at the same strain were similar among the 0-, 4-, 8-, and 12-day control sub-groups. On day 4 after application of an orthodontic force, the stress levels of the curve in the experimental sub-group were notably less than those in

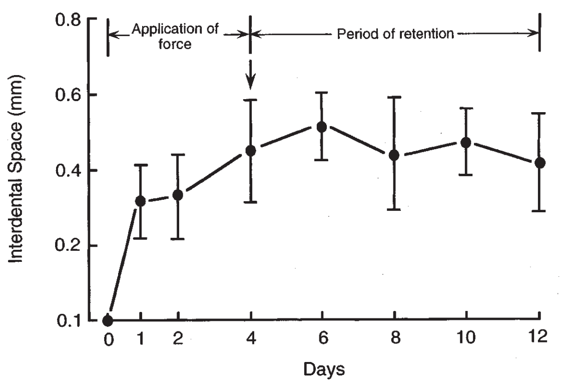

Figure 2 Changes in the interdental space obtained from the 12-day experimental sub-group during the experimental period. On day 0, an elastic band was inserted between the first and second molars. On day 4, the band was removed (arrow) and the interdental space was filled with light-cured resin that had been inserted between the teeth during the experimental period. Each point and vertical bar represent the mean ± 1 SD of nine animals (the data from one rat that died accidentally on day 8 was excluded).

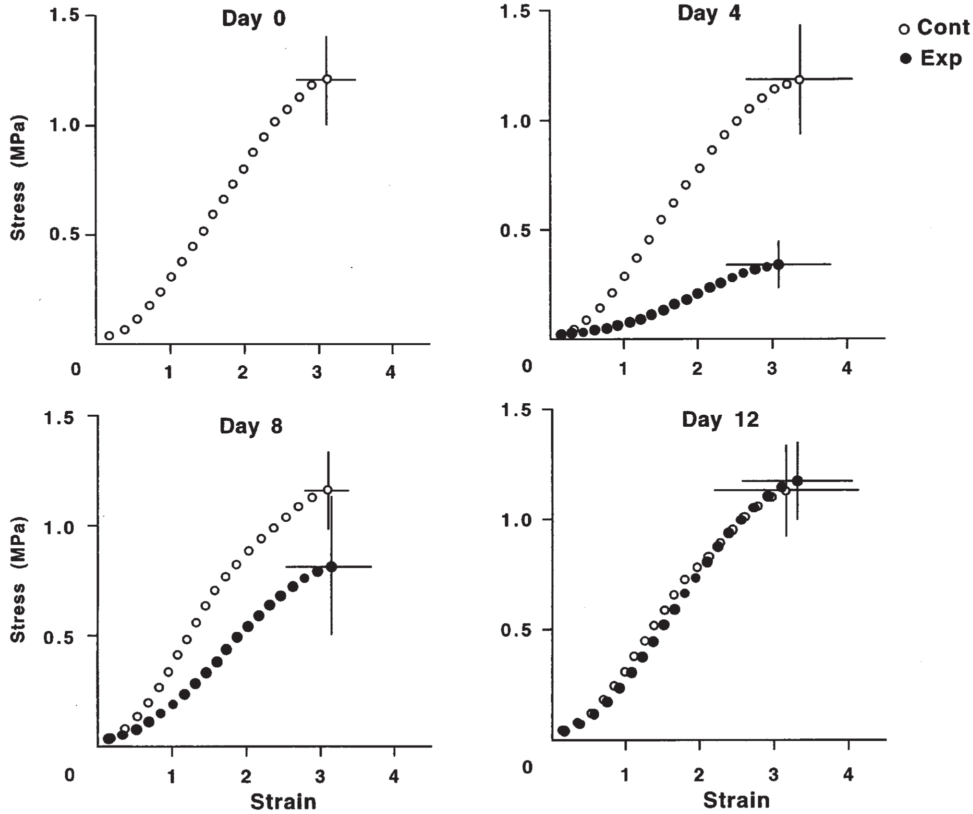

Figure 3 Stress–strain curves obtained from transverse sections of the mesial root of the rat mandibular first molar after application of an orthodontic force and after retention. On day 0, an elastic band was inserted between the teeth in all experimental sub-groups. On day 4, the band was removed and the interdental space was filled with resin. Each point represents the mean of 7–11 animals. At the peak point in each stress–strain curve, ± 1 SD for maximum shear stress and strain are shown. Cont, control sub-groups; Exp, experimental sub-groups.

the control sub-group at the same strains. On the 4th day after retention (day 8), the stress–strain curve in the experimental sub-group showed a considerable restoration. On the 8th day after retention (day 12), the shapes of stress–strain curves in the control and experimental sub-groups were almost identical.

Mechanical measurements estimated from the stress–strain curves

Figure 4 shows changes in mechanical measurements of the PDL estimated from the stress–strain curves after application of an orthodontic force and retention.

Maximum shear stress. On day 4 after application of an orthodontic force, the maximum shear stress decreased markedly and significantly ($P < 0.001$); the mean value in the 4-day experimental sub-group (0.34 MPa) was only 29 per cent of that in the control sub-group (1.18 MPa). On day 4 after retention (day 8), the maximum shear stress in the 8-day experimental group (0.81 MPa) was restored to 70 per cent of the control value (1.16 MPa) but the difference was still significant ($P < 0.05$). On the 8th day after retention (day 12), the maximum shear stress in the 12-day experimental sub-group (1.17 MPa) returned to the control level (1.13 MPa).

Maximum shear strain. The maximum shear strains in the experimental and control sub-groups were similar during the experimental period. There were no significant differences among the control or experimental sub-groups (ANOVA, $P > 0.1$) or between the experimental and respective control sub-groups at any experimental days (t-test, $P > 0.1$).

Tangent modulus and failure strain energy density. Changes in the tangent modulus (slope of the curve) and failure strain energy density were similar to those for maximum shear stress after application of an orthodontic force and retention. Both measurements decreased markedly (34 and 22 per cent of the respective control values) on the 4th day after application of an orthodontic force. On the 4th day after retention (day 8), they showed considerable restoration (62 and 64 per

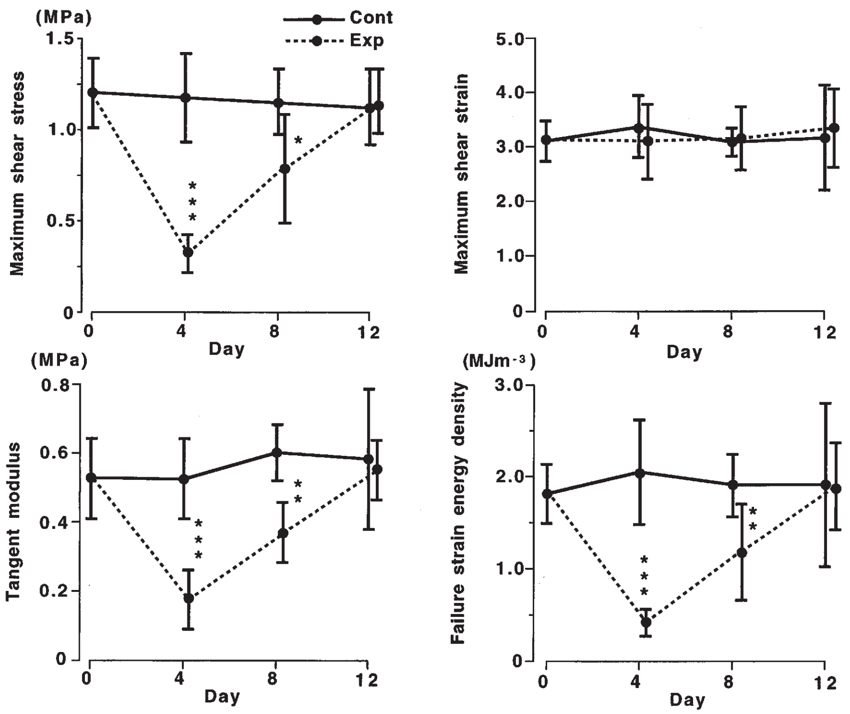

Figure 4 Changes in mechanical measurements of the periodontal ligament estimated from the stress–strain curves. Vertical bars represent the mean ± 1 SD of 7–11 animals. Significant differences between experimental and respective control sub-groups: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

cent of the respective control values). On the 8th day after retention (day 12), they returned to the control levels of 0.59 MPa and 1.91 MJm^{-3}, respectively.

Histological observations

Figure 5 shows the PDL on the mesial aspect of the mesial root of the mandibular first molar observed by light (Figure 5A,B) and polarized light microscopy (Figure 5C,D), obtained from midsagittal sections of the 12-day control (Figure 5A,C) and experimental (Figure 5B,D) animals. The periodontal space in the experimental rat (Figure 5B) was severely compressed and much narrower than that in the control rat (Figure 5A). Birefringent collagen fibres were observed running obliquely across the PDL from the cementum surface to the alveolar bone, and inserting into the alveolar bone in both the control (Figure 5C) and experimental rats (Figure 5D). However, the collagen fibres appeared to be thinner with less insertions into alveolar bone in the experimental rat than in the control.

Figure 6 shows the interdental papillae between the mandibular first and second molars observed by light (Figure 6A,B) and polarized light microscopy (Figure 6C,D), obtained from midsagittal sections of the 12-day control (Figure 6A,C) and experimental (Figure 6B,D) rats. Marked expansion of the interdental papillae (Figure 6B) and the extension of birefringent collagen fibres across the PDL (Figure 6D) were observed in the experimental rats. There appeared to be no collagen fibre insertions into some parts of the experimental rat cementum.

Discussion

In the present study, orthodontic retention was performed by placing resin into the interdental space after removal of an elastic band between the molars in rats. During the retention period, the average interdental space was quite constant. Since it has been shown that without retentive procedure the interdental space closes rapidly following removal of the elastic band (Tsuruta *et al.*, 1982; Hong *et al.*, 1992), the retention seems to be important for preventing relapse following removal of orthodontic force. Furthermore, the body and mandibular weights of the experimental animals continued to increase similarly to those of the control animals during the experimental period (Table 1). These results suggest that the harmful effects of the experimental procedures such as repeated anaesthesia, application and removal of the force, and toxicity of resin could be negligible in the present observations.

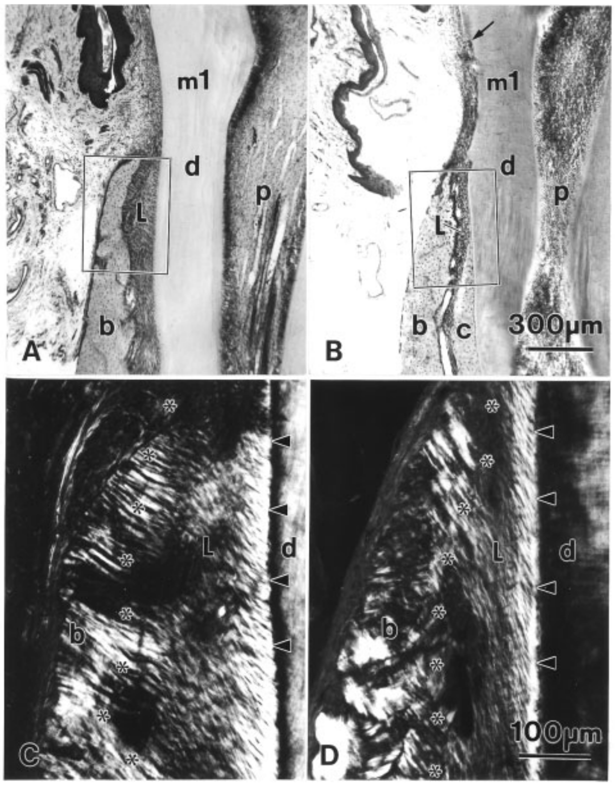

Figure 5 Midsagittal sections of the periodontal ligament of the mesial aspect of the mesial root of the mandibular first molar observed by light (A,B) and polarized light microscopy (C,D), obtained from the 12-day control (A,C) and experimental rats (B,D). The fields outlined by the rectangles in A and B were magnified (×3) in C and D, respectively. The asterisks in C and D indicate the border between the PDL and bone surface and the arrowheads the cementum surface. The arrow in B indicates root resorption. m1, mesial root of the first molar; b, alveolar bone; c, cellular cementum; d, dentine; L, periodontal ligament; p, pulp.

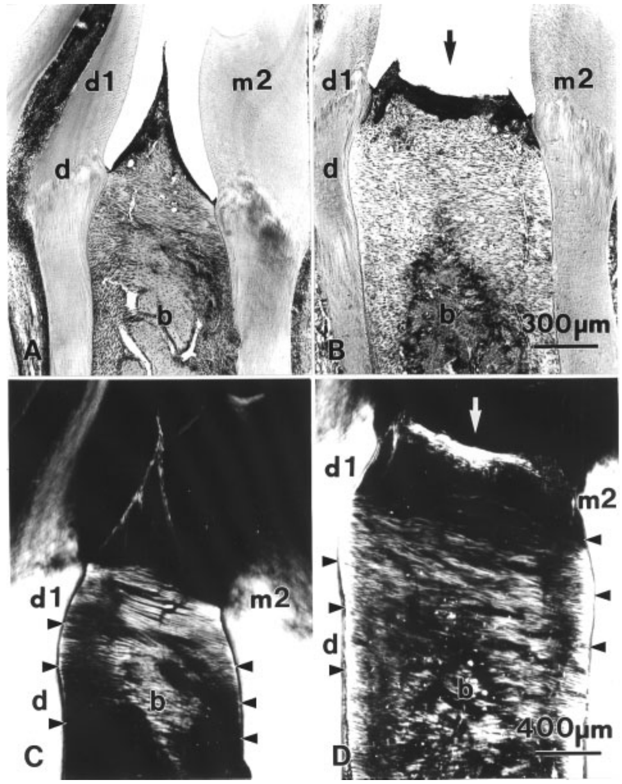

Figure 6 Midsagittal sections of the interdental papillae between the mandibular first and second molars observed by light (A,B) and polarized light microscopy (C,D), obtained from the 12-day control (A,C) and experimental (B,D) rats. The arrowheads in C and D indicate the cementum surface and the arrows in B and D the expanded papillae. d1, distal root of the first molar; m2, mesial root of the second molar; d, dentine; b, alveolar bone.

In the present investigation, the specimens for mechanical testing were kept in cold saline between killing of the animals and mechanical testing for up to 4 hours. It has been ascertained that storage of jaws in cold saline for up to 32 hours does not cause significant changes in the mechanical strength of the PDL in the rat mandibular first molar (Chiba *et al*., 1982). Thus the effects of degenerative changes in the periodontal tissues on the mechanical properties of the PDL seem to be small and negligible under the present experimental conditions.

It was found that the maximum shear stress, tangent modulus, and failure strain energy density decreased markedly on the 4th day after force application and restored completely on the 8th day after orthodontic retention (Figures 3 and 4). Similar mechanical responses of the PDL have also been demonstrated by analyses of load–deformation curves, when a whole tooth was extracted from its socket in the rat mandibular (Tsuruta *et al*., 1982) or maxillary (Hong *et al*., 1992) first molars. These data suggest that 8 days of retention are sufficient to restore the mechanical strength of the PDL, which had been severely impaired by the application of an orthodontic force in the rat mandibular first molar.

Numerous studies have shown that tissue damage such as disruption of collagen fibres (Zaki and Van Huysen, 1963; Reitan, 1967; Azuma, 1970; Kuitert *et al*., 1988/89), compression and expansion of the PDL (Reitan, 1967; Hong, 1990), and resorption of bone and tooth (Zaki and Van Huysen, 1963; Reitan, 1964, 1967; Tanaka *et al*., 1990) occur following application of orthodontic force. Severe compression and expansion of the PDL, and resorption of bone and tooth were still observed in the experimental rats on the 8th day after retention (Figures 5 and 6). However, birefringent collagen fibres running across the compressed and extended PDL in the experimental rats were observed although the periodontal collagen fibres appeared to be thinner with less insertions into alveolar bone or cementum as compared with the control rats (Figures 5 and 6). This suggests that collagen fibres in the PDL impaired by orthodontic force application can partially rearrange and reorganize within the 8 day retention period.

There have been several reports showing that the turnover rate of collagens is faster in the periodontal tissues than in other connective tissues (Sodek, 1977, 1989; Imberman *et al*., 1986; Sodek and Ferrier, 1988). It has been reported that the half-life for collagens in the periodontal tissues of rat molars is less than 3 days, which is approximately 7 days shorter than that in lamina propria of gingival tissues (Sodek, 1977, 1989; Sodek and Ferrier, 1988). Imberman *et al.* (1986) also observed a much shorter collagen half-life in the periodontal tissues (8.8 days) than in the skin (50 days) and palatal mucosa (21 days) in rats. Thus, the shorter half-life for collagen in the periodontal tissues of rat molars supports the view that reorganization and rearrangement of impaired periodontal collagen fibres occurred rapidly to some extent during the 8 days of retention in the present investigation.

It has recently been demonstrated that periodontal collagen fibres play the main role in bearing the mechanical properties in rat molar teeth (Kawada and Komatsu, 2000). The restoration of the mechanical properties in the impaired PDL, thus, seem to be mainly dependent on the reorganization and rearrangement of the periodontal collagen fibres. However, although in the present investigation the reorganization of periodontal collagen was incomplete, the biomechanical properties of the PDL were completely restored after 8 days of retention. This suggests that other factors besides periodontal collagen fibres play a role in supporting the mechanical properties in rat molar teeth. It has been shown that interfibrillar substances such as proteoglycans are also important in maintaining the structure and function of rat molar PDL (Watanabe and Komatsu, 1997; Kanazashi and Komatsu, 1999). This seems to support the present view. However, to understand the involvement of interfibrillar substances in the restoration of mechanical properties during retention in rat molar teeth, further studies are necessary.

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