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**DENTAL TRAUMATOLOGY**

# The biomechanical properties of the healing periodontium of replanted rat mandibular incisors

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Abstract – One of the most important aspects in tooth replantation seems to be restoration of the tooth support function of the healing periodontal ligament (PDL). We examined the support function, as measured by the mechanical properties, of the healing PDL at 7, 14, and 21 days after replantation of the left mandibular incisor in rats. From each dissected left mandible, a transverse section(650 μm in thickness) of the incisor was cut through an axis near the labial alveolar crest. Each section was intrusively loaded at a rate of 5 mm $min^{-1}$, and the shear stress–strain curve for the PDL was analyzed. Mechanical measures of the healing PDL showed gradual improvement after replantation. By 21 days, the mechanical strength returned to 53% of the control value; the extensibility, to 85%; the stiffness, to 61%; and the toughness, to 52%. The healing PDL exhibited reattachment of fibers in the middle region of the PDL, and the birefringent collagen fibers appeared to have regained the functional orientation by 14 days. The ratios occupied by the birefringent collagen fibers in the tooth-related, middle, and bone-related areas of the healing PDL gradually improved and returned to 78, 51, and 48% of the respective control values by 21 days. These results suggest that the support function of the healing PDL is gradually restored and that the biomechanical restoration is closely related to the reorganization and reorientation of collagen fiber bundles in replanted rat incisors.

Key words: rat incisor; replantation; periodontium; biomechanics; collagen fibers

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There have been many reports on the healing processes of the periodontal ligament (PDL) after tooth replantation in dogs (1–3), monkeys (1, 4), rats (5–9), and ferrets (10). However, the tooth support function of the healing PDL after tooth replantation has not yet been examined. It has been pointed out that one of the most important aspects in tooth replantation is restoration of the support function of the healing PDL (11–13), as in other healing connective tissues (14, 15).

The support function of the PDL of the rat incisor has widely been assessed by analyzing the load–deformation curves when the tooth was extracted from the dissected jaw (16, 17) and by analyzing the stress–strain curve when the tooth was pushed out of surrounding alveolar bones using a sliced specimen (18–20). The rat incisor is continuously erupting, and its PDL may differ markedly from that of rooted tooth. However, experimental procedures of tooth replantation seem to be simple because the shape of the rat incisor is cylindrical.

Using polarized light microscopy, it has been shown that the mechanical properties of the normal PDL are largely dependent upon the arrangement and organization of collagen fibers (21–23). Polarized light microscopic analyses of collagen fibers have effectively been made to understand the processes of wound healing in guinea pig skin (24) and rabbit cornea (25), but not in the healing PDL of experimental animals.

The main purposes of the present study were to obtain a simple experimental model to study the restoration process of the mechanical properties of the healing PDL at different intervals after tooth replantation using the rat mandibular incisor, and then to elucidate a relation between the mechanical and morphologic restorations.

## Materials and methods

### Extraction–replantation procedure of the rat mandibular incisor

Seventy male Wistar rats, aged 5 weeks, were fed a powdered diet (CE-2, Nippon Clea, Tokyo, Japan) and given water *ad libitum* during the experimental period. To facilitate extraction of the rat mandibular incisor from its socket without serious damage to the tooth and alveolar bones, the left mandibular incisor was shortened at the gum level three times repeatedly at 2-day intervals; the repeated shortenings of rat incisors markedly reduce the mechanical strength of the PDL (17, 18). The repeated shortenings may cause exposure of the pulpal soft tissues, but no subsequent pulpal infection and inflammation have been detected in our previous studies (17, 18, 26). The rats were divided into four control and three experimental subgroups of 10 animals each. One day after the last shortening of the incisors, the left mandibular incisors of rats in the experimental subgroups were extracted (Fig. 1a) using extraction forceps. The extracted incisor (Fig. 1b) was rinsed with phosphate-buffered saline (PBS, pH 7.3), and its odontogenic base was removed. Then, the incisor was put back (replanted) into its socket (Fig. 1c). This procedure was carried out under anesthesia with intraperitoneal injection of pentobarbital (50 mg kg$^{-1}$ body weight; Nembutal, Abbott, Chicago, USA). There was about 5-min difference between the extraction and the replantation. In the control animals, their left mandibular incisors were also shortened as in the experimental animals, but they were not extracted. All rats in the experimental subgroups were subcutaneously injected with antibiotic at 10 mg kg$^{-1}$ body weight (Tylocin®, Eranko, USA) immediately after replantation.

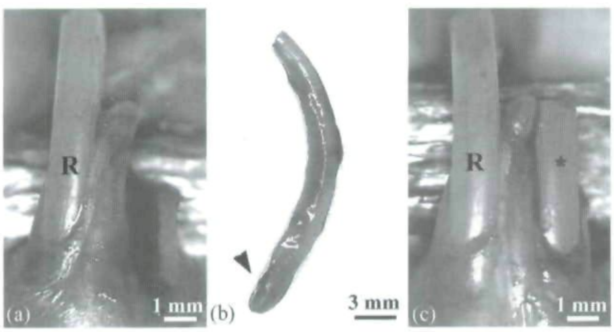


*Fig. 1.* Photographs of the labial aspect of the mandible after tooth extraction (a), extracted left mandibular incisor (b), and labial aspect of the mandible after tooth replantation (c) in an experimental rat. The arrowhead indicates the odontogenic base of the extracted incisor. The asterisk indicates the replanted incisor. R, right intact incisor.

### Preparation of mechanical specimens

Experimental subgroups of animals were killed with an overdose of ether at 7, 14, and 21 days after replantation. Control subgroups of animals were similarly killed on the same days. Immediately after death, their mandibles were dissected, and the adherent soft tissues were removed. From each dissected left mandible, a transverse section (about 0.65 mm in thickness) of the incisor with its surrounding PDL and alveolar bones was cut through the axis near the labial alveolar crest (Fig. 2) using a bone saw (Isomet, Buehler, IL, USA), with special care to avoid tissue damage (18). The thickness of sections was measured with a dial thickness gauge (Peacock, Tokyo, Japan). The

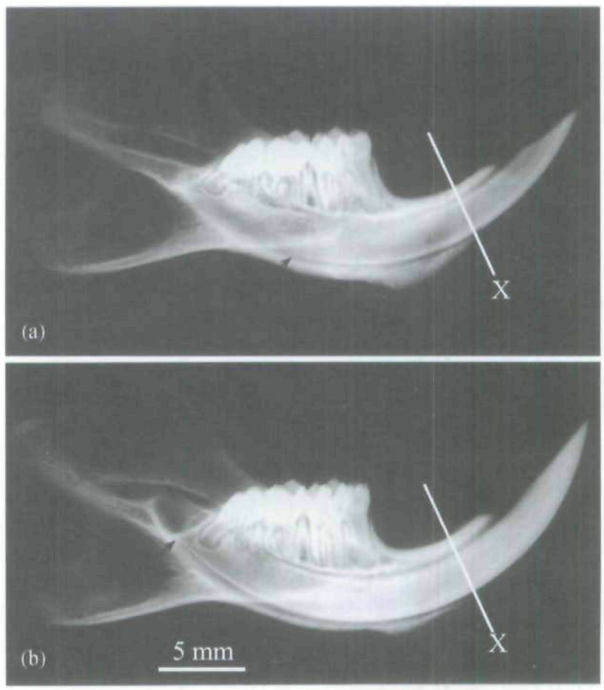


*Fig. 2.* Radiographs of the left mandibles of an experimental rat (a) at 21 days after replantation of the incisor, and of a control rat (b). Transverse sections (0.65 mm in thickness) of the incisors with their surrounding periodontal ligament and alveolar bones were cut through the axes (X). Arrows indicate the proximal ends of the labial incisor dentin.

sections were kept in PBS at 4°C until mechanical testing was performed to minimize the post-mortem proteolytic degradation of the tissues.

### Radiographic analyses

Radiographs of the sections were taken in a soft X-ray apparatus and were processed in an image analyzer (Luzex 3U, Nikon, Tokyo, Japan). We then measured the circumferences of the tooth and socket wall, the perimeters of the lingual cementum and socket wall between the enamel/cementum junctions on both sides of the tooth, and the sectional area of the lingual PDL. We also calculated the area of the PDL facing the cementum and the average width of the PDL (18, 19).

### Mechanical testing

The method of mechanical testing has previously been described in detail (18, 19). In brief, the bony part of the section was clamped between two plates of a sample holder, and the holder with the specimen was placed in a chamber mounted on a testing machine. The chamber was filled with PBS. The tooth component was then loaded intrusively at a velocity of 5 mm $min^{-1}$ until failure, and a load–deformation curve was recorded. The mechanical testing was performed at a temperature of 23–25°C. The time between killing the animals and mechanical testing ranged from 57 to 196 min.

Shear stress values were calculated by dividing the load values by the area of the PDL facing cementum. Shear strains were calculated by dividing the deformations by the average width of the PDL. Thus, the load–deformation curve was transformed into a shear stress–strain curve. From each stress–strain curve, the following biomechanical measures were estimated (18, 19, 27): maximum shear stress, maximum shear strain, tangent modulus, and failure strain energy density.

### Morphologic examinations

From the remaining part of the left mandible in each rat, we also obtained a transverse section (about 0.65 mm in thickness) adjacent to the mechanical specimen. The sections were fixed in neutral buffered formalin, demineralized in 14.5% EDTA (pH 7.2) for 2 weeks at 4°C, and embedded in gelatin. Frozen sections were cut transversely with a microtome (1320, Leitz, Germany) setting of 20 μm, stained with hematoxylin and eosin, and mounted in glycerin jelly. Sections were observed microscopically under ordinary transmitted or polarized light (Laborlux 12 Pol S, Leitz, Germany). Five to eight rats in the control and experimental subgroups were used for morphologic examinations.

In each section, a light microscopic photograph of the lingual PDL was taken. On the photographic print, the numbers of periodontal fibroblasts were counted in three rectangular areas (30 μm in depth, 72 μm in width): the tooth-related, middle, and bone-related areas (20, 28). A polarized light microscopic photograph of the same site in each specimen was also taken and digitized according to the method described previously (23). The ratios occupied by birefringent collagen fibers were estimated (23) in the tooth-related, middle, and bone-related areas.

### Statistics

The differences of the mean values were examined by the Student's $t$-test and, when appropriate, by Scheffé's test (multiple comparison). One-way analysis of variance (ANOVA) was used to examine differences in the radiographic and mechanical measures at 7, 14, and 21 days after replantation.

## Results

### General observations

The mean body weights of the experimental subgroups did not show significant differences from those of the control subgroups at 7, 14, and 21 days after replantation. The mean weights of left mandibles in the experimental subgroups did not show significant differences from those in the control subgroups at 7 and 14 days, but the mean weight was significantly less in the experimental subgroup than in the controls at 21 days ($t$-test, $P < 0.01$).

### Radiographic observations

In the experimental rat at 21 days after replantation, the proximal end of the replanted incisor dentin had moved toward the incisal direction and was located just below the distal root of the first molar (Fig. 2a). In the control rat, the proximal end of the incisor dentin was extended below and beyond the distal root of the third molar (Fig. 2b). The replanted incisor had a chisel-shaped, incisal edge (Fig. 2a) like the control tooth (Fig. 2b), but the bevel angles seemed to be different. The labial side of the mandibular bone in the experimental rat appeared to be thicker than that in the control rat.

The perimeters of the cementum and socket bone in the control subgroups increased gradually from 0 to 21 days (ANOVA, $P < 0.001$), but those in the experimental subgroups did not show significant increases during the experimental period. The average width of the PDL in the experimental subgroup was less than that in the control subgroup at 7 days ($t$-test, $P < 0.05$) but did not differ from the controls at 14 and 21 days.

### Stress–strain curves of the PDL

Figure 3 shows the stress–strain curves of the PDL obtained from the transverse sections of the left mandibular incisors after replantation. The graph shows only the rising parts of stress–strain curves. The stress–strain curves obtained from the control and experimental subgroups were both non-linear and sigmoidal in shape. At 7 days, the stress levels in the experimental subgroup were markedly less than those in the control subgroup at the same strain levels. At 14 and 21 days, the slopes of stress–strain curves in the experimental subgroups were similar to those in the controls, but the mean maximum stresses and strains were less in the experimental subgroups than in the control subgroups.

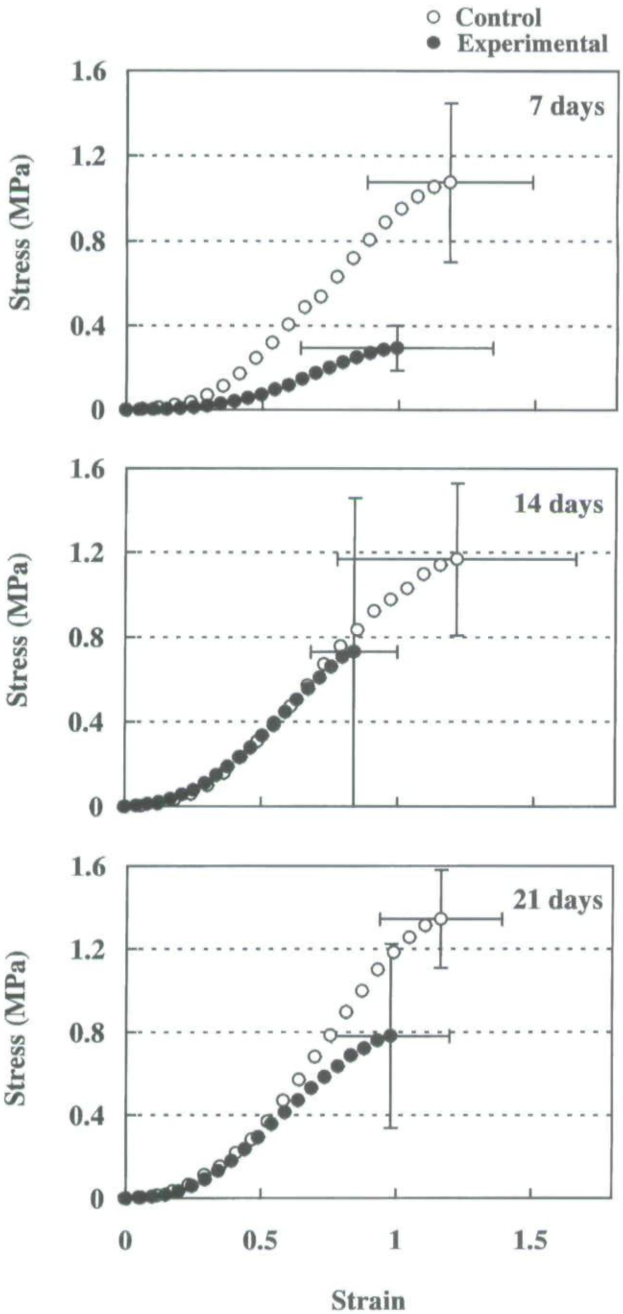


*Fig. 3.* Stress–strain curves of the periodontal ligament obtained from transverse sections of the mandibular incisors at 7, 14, and 21 days after replantation in the control and experimental subgroups. Each point represents the mean of 10 rats. Vertical and horizontal bars represent ±1 SD for the maximum shear stress and strain, respectively.

### Biomechanical measures of the PDL

Figure 4 shows changes in the biomechanical measures estimated from the stress–strain curves of the PDL after tooth replantation.

- *Maximum shear stress*: The mean values in the control subgroups increased gradually during the experimental period, but not significantly (ANOVA, $P > 0.1$). The mean value in the experimental subgroup at 7 days after replantation decreased markedly to 30% of the control value (*t*-test, $P < 0.001$). Then, the mean values in the experimental subgroups increased at 14 days but did not change from 14 to 21 days, the mean values being 63 and 53% of the respective control values at 14 and 21 days. The difference between the experimental and control subgroups at 21 days was significant (*t*-test, $P < 0.01$).
- *Maximum shear strain*: While the mean values were less in the experimental subgroups than in the control subgroups during the experimental period, the difference was only significant at 14 days (*t*-test, $P < 0.05$). The mean values in the experimental subgroups were 83, 68, and 85% of those in the respective control subgroups at 7, 14, and 21 days.
- *Tangent modulus:* The mean values in the control subgroups increased significantly during the experimental period (ANOVA, $P < 0.05$). The mean value in the experimental subgroup at 7 days decreased markedly to 48% of the control value (*t*-test, $P < 0.01$). The mean values in the experimental subgroups gradually increased from 7 to 21 days (not significant, ANOVA), the values being 74 and 61% of the respective control values at 14 and 21 days. The difference was significant between the experimental and control subgroups at 21 days (*t*-test, $P < 0.01$).
- *Failure strain energy density*: The mean values in the control subgroups increased gradually during the experimental period, but not significantly (ANOVA, $P > 0.1$). The mean value in the experimental subgroup at 7 days decreased markedly to 20% of the control value (*t*-test, $P < 0.001$). The mean values in the experimental subgroups gradually increased from 7 to 21 days, but not significantly (ANOVA, $P > 0.1$), the values being 42 and 52% of the respective control values at 14 and 21 days. The difference was significant between the experimental and control subgroups at 21 days (*t*-test, $P < 0.01$).

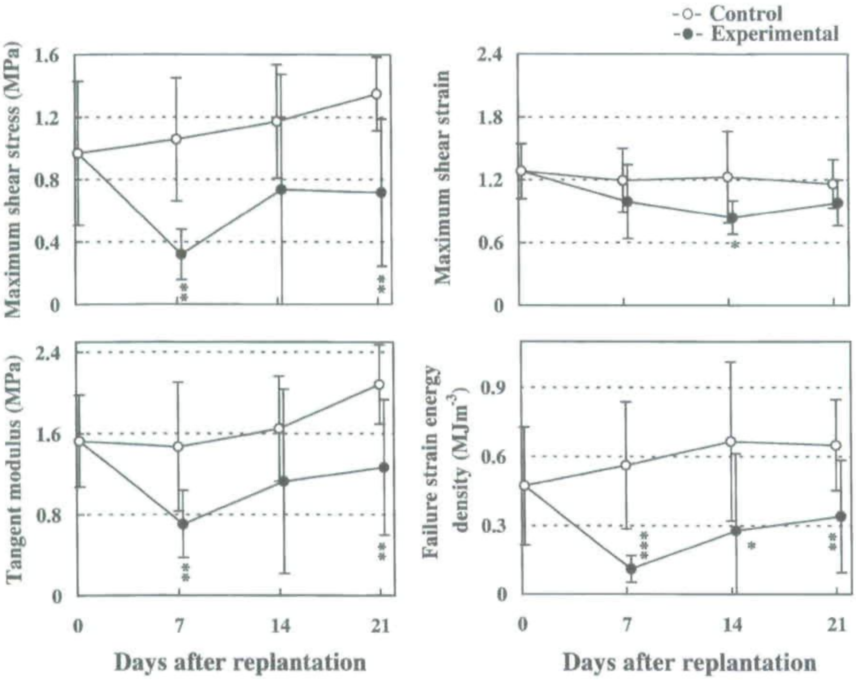


*Fig. 4.* Biomechanical measures of the periodontal ligament estimated from the stress–strain curves shown in Fig. 3. Each point and the vertical bars represent the mean and ±1 SD of 10 rats. Significant differences from the respective controls (*t*-test): $^{*}P < 0.05$, $^{**}P < 0.01$; $^{***}P < 0.001$.

In general, the maximum shear stress, tangent modulus, and failure strain energy density showed similar patterns of restoration after replantation, but the maximum shear strain showed relatively small changes during the experimental period.

### Light and polarized light microscopic observations

Figure 5 shows light micrographs of the lingual PDL in transverse sections of the mandibular incisors in the experimental rats at 7 (Fig. 5a), 14(Fig. 5b), and 21(Fig. 5c) days after replantation and in a 21-day control rat (Fig. 5d). Hematoxylin and eosin-stained section obtained from the control rat showed a high density of PDL cells (Fig. 5d). These cells appeared to be located in the spaces between the fiber bundles. Blood vessels were mainly located near the bone surface. At 7 days after replantation, reattachment of the PDL cells was observed in the middle region of the ligament; the site of rupture was determined by the observation of sections obtained just after replantation (Fig. 5a). Nuclei of the PDL cells around the approximate site of reattachment were round in shape and not oriented regularly. The general orientation of the periodontal fibroblasts showed almost normal appearances at 14 and 21 days, as compared with the 21-day control (Fig. 5d). Blood vessels were predominately observed in the bone-related area in all specimens.

Figure 6 shows polarized light micrographs taken from the same sites of the sections shown in Fig. 5. In the control section, birefringent collagen fiber bundles running between the cementum and bone surfaces were clearly observed (Fig. 6d). Birefringent collagen fiber bundles in the bone-related area were thick and wavy. In the tooth-related area, numerous birefringent fiber bundles were extended from the cementum surface. In the intermediate area, thinner birefringent fibers were seen. At 7 days after replantation (Fig. 6a), thick birefringent fiber bundles were seen extending out from the alveolar bone, although they were not distinctly wavy. In the intermediate area, there appeared to be no continuity of birefringent PDL fibers between the bone and cementum at the approximate site of reattachment, but thin fibers with loose structure and weak birefringence were observed (Fig. 6a). In the tooth-related area, short birefringent fibers were seen. The birefringent fiber bundles extending out from the alveolar bone appeared to join together (become thicker) and appeared wavy again at 14 days (Fig. 6b). The arrangements of birefringent fibers in the intermediate and tooth-related areas were still irregular. Birefringent fiber bundles running from the cementum to bone surfaces were observed at 21 days (Fig. 6c). While the fiber bundles showed a distinct waviness and were well organized, they still appeared to be thinner with less intensity of birefringence as compared with the 21-day control (Fig. 6d).

#### *Image analysis of the PDL*

The mean numbers of PDL cells were significantly less in the experimental subgroups than in the control subgroups in the bone-related area at 7 days (*t*-test, $P < 0.05$) and in the tooth-related area at 7 (*t*-test, $P < 0.001$) and 14 (*t*-test, $P < 0.01$) days. In the middle area at 7 and

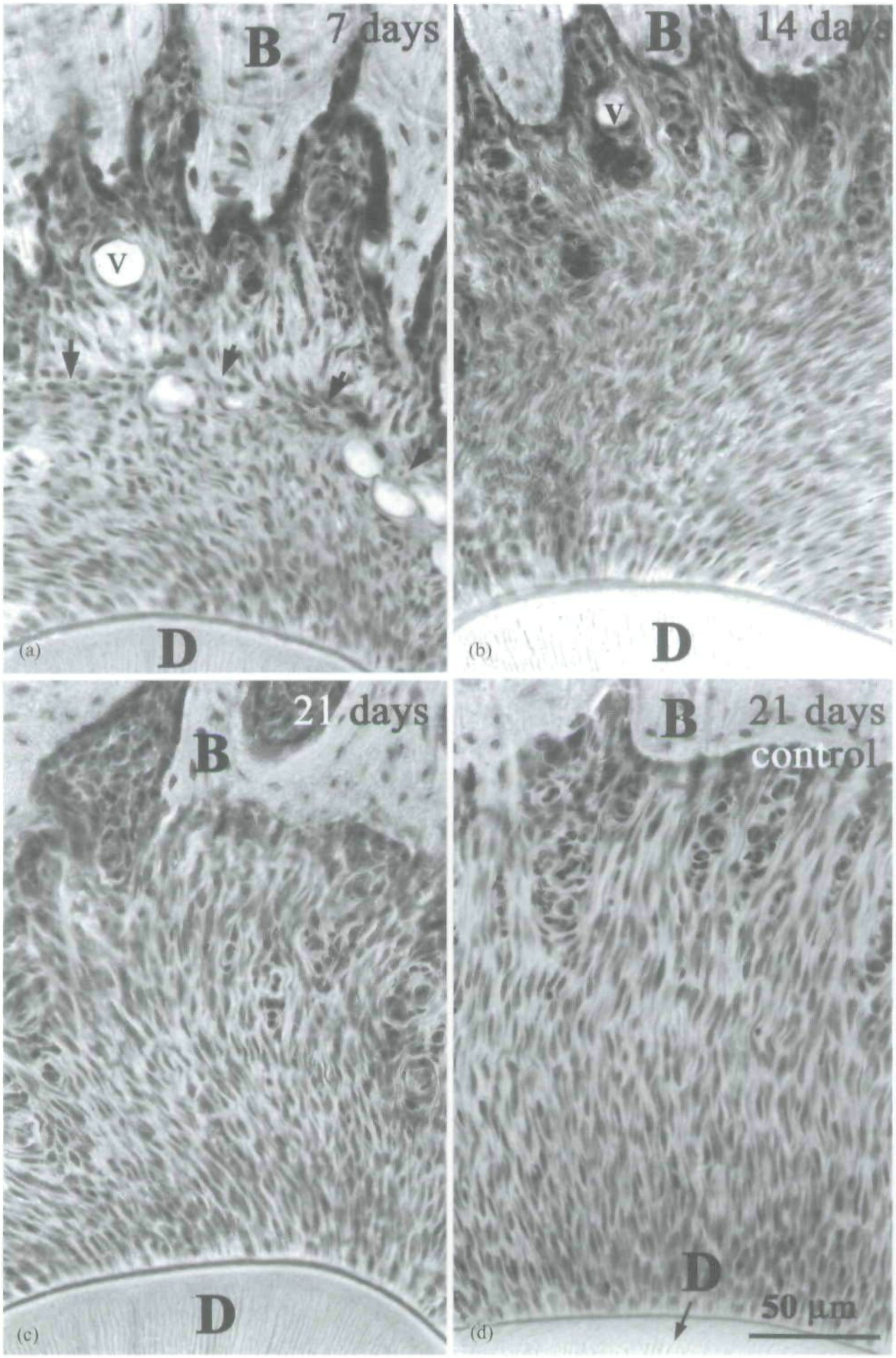


*Fig. 5.* Light micrographs of the lingual periodontal ligament (PDL) in transverse sections of the mandibular incisors in the experimental rats at 7 (a), 14 (b), and 21 (c) days after replantation, and in a 21-day control rat (d). Arrows (a) indicate the approximate site of reattachment of the PDL, which was determined by the observation of sections obtained just after replantation. B, alveolar bone; D, dentin; v, blood vessel. Hematoxylin and eosin.

14 days and in all areas at 21 days, significant differences between the experimental and control subgroups were not found.

Figure 7 shows the ratios occupied by birefringent collagen fibers in the bone-related, middle, and tooth-related areas of the PDL after replantation in the control and experimental subgroups. The mean ratios in the experimental subgroups were significantly less than those in the control subgroups at 7, 14, and 21 days in all three areas (*t*-test,

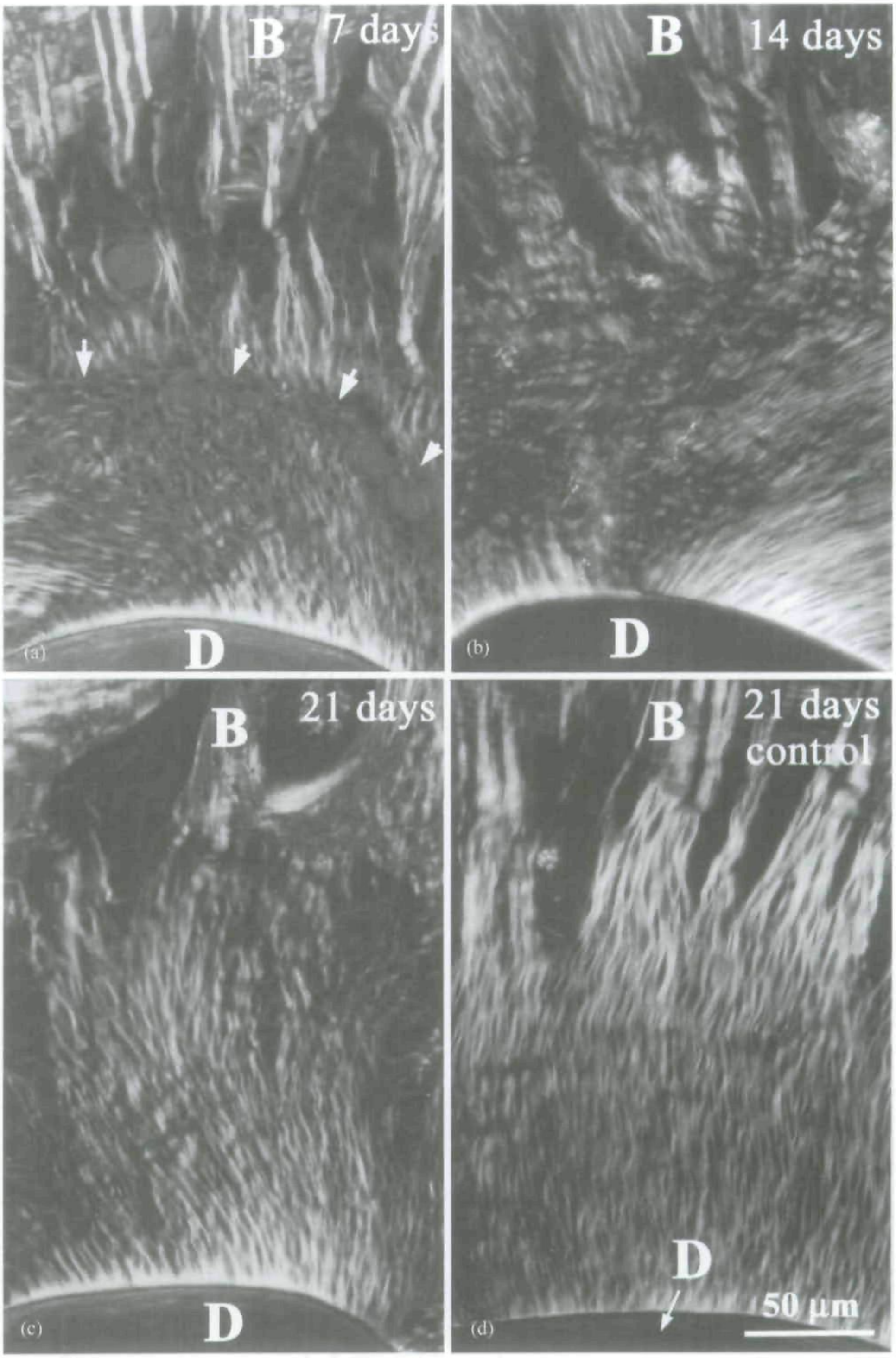


*Fig. 6.* Polarized light micrographs taken from the same sites of the sections shown in Fig. 5, that is, the lingual periodontal ligament (PDL) of incisors in the experimental rats at 7 (a), 14 (b), and 21 (c) days after replantation, and in the 21-day control rat (d). Arrows (a) indicate the approximate site of reattachment of the PDL. B, alveolar bone; D, dentin.

$P < 0.05$–$0.01$), except for the middle and tooth-related areas at 14 days. The mean ratios in the experimental subgroups were 48, 51, and 78% of the control values in the bone-related, middle, and tooth-related areas, respectively, at 21 days.

*Resorption of teeth*

At 7 days after replantation, resorption of incisor dentin was not observed in any of the five sections examined. Small and shallow resorption lacunae were observed mainly on the buccal side in three out of eight replanted incisors at 14 days and in four out of seven replanted incisors at 21 days. A deep resorption lacuna was found on the buccal side of one replanted incisor at 21 days. There was no indication of ankylosis in any of the specimens.

## Discussion

The present study evaluated the restoration process of the support function of the healing PDL in replanted incisors by measuring the stress–strain curve of the healing PDL. The four mechanical measures estimated from the curve may provide useful information (14, 18, 19, 27). The maximum shear stress and maximum shear strain reflect the mechanical strength and extensibility of the PDL, respectively. The tangent modulus is used as a measure for the stiffness of the straightened-out collagen fiber bundles in the PDL (21, 23). The failure strain energy density indicates the energy required to break the specimen.

The mechanical properties of the healing PDL were gradually restored from 7 to 21 days after replantation of the rat mandibular incisor. The mean values for the mechanical strength, extensibility, stiffness, and toughness of the healing PDL were 53, 85, 61, and 52%, respectively, of the control values at 21 days. These values are roughly in agreement with the restorations in the injured PDL after extrusive luxation of upper central incisors in vervet monkeys in which the mean values for the mechanical strength, extensibility, stiffness, and toughness have been restored to 62, 62, 92, and 51%, respectively, at 2 weeks (29). It is suggested that there are no fundamental differences between monkey and rat teeth in the restoration processes of healing PDL from the biomechanical point of view.

There have been many reports on the restoration of mechanical properties in other connective tissues after injury. For example, the restoration rates of the breaking loads of the healing tendons in rabbits attained 2, 7, 13, 38–60, and 67–70% at 7, 14, 28, 56–98, and 168–280 days, respectively, after injury (30, 31). The restoration rates of the tensile strengths of the healing skin wounds in guinea pigs exhibited 2, 12, 15, 25, 76, and 83% at 6, 15, 21, 28, 45, and 180 days, respectively, after incision (24). The restoration rates of the breaking loads of the healing skin wounds in rats exhibited 6 and 18% at 10 and 20 days, respectively, after incision (32, 33). The mechanical strengths of the healing PDLs returned to about 50% of the control values at 2–3 weeks after injury in the present and previous (29) studies. It seems that, in terms of the biomechanics, the healing rate of the PDL could be more rapid than that of other connective tissues. Such a rapid healing rate may be related to the rapid turnover rate of collagens (29). In fact, it has been demonstrated that the half-lives of mature, neutral salt-insoluble collagen are 1–8.8 days in the rat molar PDL, 6–7.8 days in the rat incisor PDL, and 15–50 days in rat skin (34–36).

Polarized light microscopic analysis has been useful for investigating the macromolecular orientation and organization of birefringent collagen fibers in connective tissues (24, 25, 37). In the present study, functional reorientation and increased organization of birefringent PDL collagen fibers were found after tooth replantation (Fig. 6). Furthermore, image analysis showed gradual increases in the areas

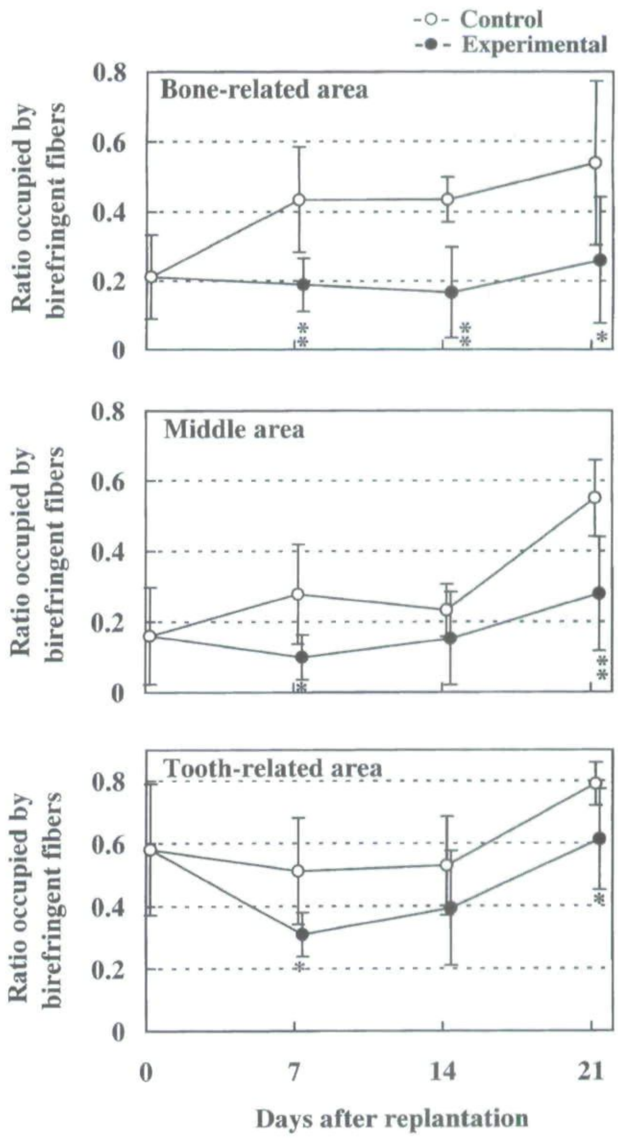


*Fig. 7.* The ratios occupied by birefringent fibers in the tooth-related, middle, and bone-related areas of the periodontal ligament at 0, 7, 14, and 21 days after replantation. Each point and the vertical bars represent the mean and ±1 SD of five to eight rats. Significant differences from the respective controls (*t*-test): $^{*}P < 0.05$; $^{**}P < 0.01$.

occupied by birefringent PDL collagen fibers (Fig. 7), the mean values being 50–70% of the control values at 21 days, which were almost identical to the biomechanical restorations seen in the healing PDL (Fig. 4). On the other hand, the numbers of PDL cells did not correspond to the mechanical restorations. Therefore, we suggest that the restoration of the mechanical properties of the healing PDL is closely related to the degree of reorganization and functional reorientation of the collagen fibers, but not necessarily to the number of PDL cells.

Normal functional teeth are known to have better biomechanical properties of the PDL than hypofunctional teeth (17, 18, 26, 38). In the present study, the replanted teeth erupted and gradually showed sharp and chisel-shaped incisal edges in accordance with the recovery of attritional activities. The chisel shape of the incisal edge reflects the functional wear by attrition with the opponent teeth. It is plausible that the recovery of attrition induces better restoration of the mechanical strength and reorganization of collagen fibers of the healing PDL. Indeed, when the total amount of eruption of replanted incisors was greater, so was the recovery of the mechanical strength of the healing PDL (Komatsu et al., unpublished). Thus, we suggest that intermittent mechanical stimuli such as occlusal contacts play important roles in the reorganization and functional reorientation of collagen fiber bundles, and in the restoration of the mechanical strength of the healing PDL.

It has been pointed out that the main problems in tooth replantation are resorption and ankylosis of the bones and teeth (10, 11, 39). In the present study, ankylosis of the bones was not detected and there was no severe resorption of teeth and bones except for a deep resorption lacuna of a tooth found at 21 days, which could have been caused by pulpal infection and subsequent inflammation during the experimental period.

Another problem of using the rat incisor as an experimental model for tooth replantation is that as the replanted incisors still continue to erupt slowly, the period of observation is limited and the PDL of continuously erupting incisors may differ from that of teeth of limited growth. On the other hand, it has been shown that the shape of the stress–strain curve of the rat incisor PDL is similar to that of the PDL of teeth of limited growth (19, 27, 40–42). Therefore, it is possible that biomechanical properties of the healing PDL in replanted rat incisors may be similar to those in the replanted teeth of limited growth, and provide basic information on the restoration process of the support function of both types of teeth. This model may also be useful because the experimental procedures are simple and straightforward. In addition, rats are economical compared with other animals such as dogs and monkeys (6).

An interesting future project would be to examine the effects of various growth factors that may accelerate regeneration of the PDL and of drugs such as inhibitors of bone resorption on the restoration of the mechanical properties of healing PDL after tooth replantation.

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