Remodeling mechanisms of transseptal fibers during and after tooth movement

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Remodeling and rearrangement of supra-alveolar tissue are indispensable for the stability of the dentition after orthodontic tooth movement. Many authors have studied the reconstruction of alveolar bone and the rearrangement of periodontal membrane. They found that collagen synthetic activity increased rapidly depending on the orthodontic stimuli,[1-5] and that remodeling and rearrangement of periodontal membrane is nearly completed in the first few days.[1-3]

However, Reitan,[6,7] Erikson,[8] and Thompson[9,10] reported that transseptal fibers in supra-alveolar tissue did not show rearrangement and remodeling even after long-term retention. Therefore, surgical transection of the supra-alveolar tissue was recommended to reduce the tendency for relapse.[11-15]

Minkoff[16] and Rippin[17,18] used autoradiography to study protein turnover rates of subcrestal and supracrestal fibers. They stated that fibroblast activity was lower in dento-gingival regions than in dentoalveolar and transseptal regions, and was lower in transseptal regions than in dento-alveolar regions. In addition, they found that the half-life of collagen fibers was 5.7 days in dento-alveolar regions, 8.4 days in transseptal regions, and 25 days in dento-gingival regions. Boisson[5] reported that collagen synthetic activity increased by 70% in transseptal fibers and by 300% in periodontal membrane during tooth movement.

Abstract

The remodeling mechanisms of transseptal fibers during and after tooth movement were investigated histologically. An autoradiographic study was conducted to assess the synthetic response. One hundred fifty male Wistar strain growing rats were divided into three groups—one control and two experimental groups. Animals in the experimental groups were subjected to tooth movement with 25 g and 150 g of force. Maxillary first and second molars were separated and retained mechanically.

Transseptal fibers were stretched in proportion to the amount of force applied for up to 2 days. Dynamic remodeling with proliferation of fibroblasts during tooth movement and slow rearrangement during retention periods occurred in both experimental groups. Collagen phagocytosis within the fibroblasts was observed ultrastructually during the experimental periods. The number of silver grains in fibroblasts in the collagen fibers increased 160% the first day and 206% during the first 3 days.

Proliferating fibroblasts remodeled the transseptal fibers through the synthesization and degradation of collagen fibers.

Key Words

Remodeling mechanisms • Transseptal fibers •Tooth movement

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Table 1
Classification and number of animals

	During tooth movement					After tooth movement		
	1 day	3 days	5 days	7 days	10 days	10 days	30 days	60 days
Light microscopy								
Control group	5	5	5	5	5	5	5	5
Light force	5	5	5	5	5	5	5	5
Heavy force	3	3	3	3	3	3	3	3
Electron microscopy								
Control group	2	2	2	-	2	2	2	2
Heavy force	2	2	2	-	2	2	2	2
Autoradioagraphy								
Control	3	3	3	-	-	-	-	-
Heavy force	3	3	3	-	-	-	-	-

The purpose of this study was to investigate the changes in collagen synthetic activity and the remodeling mechanisms of transseptal fibers during and after tooth movement. For this purpose, morphological characteristics of collagen fibers and fibroblasts were studied with light and electron microscopy, and synthetic activity of fibroblasts was examined autoradiographically.

Materials and Methods

Experimental animals and orthodontic appliances

One hundred fifty male Wistar strain rats (6 weeks old, 140 gm average body weight) were used for this experiment. The animals were divided into a control and two experimental groups based on the degree of force which was applied for tooth movement (Table 1). A 0.5 mm-deep hole was made on the lingual surface of the first and second left maxillary molars. An expansion spring was placed in the hole for 1, 3, 5, 7 and 10 days to expand the intermolar space (Figure 1). The expansion spring was constructed with .012 and .018 orthodontic round wire with a helical loop and two arms. The spring was activated 1.0 mm and exerted 25 g (light force group) and 150 g (heavy force group) of force (Figure 2). After tooth movement, the first and second molars were retained with .018 round wire for 10, 30 and 60 days in order to maintain the intermolar space. All procedures were carried out under general anesthesia using Nembutal (Abbott, U.S.A).

Histological study

Light microscopy

Animals of each group were sacrificed at each experimental period (Table 1), and the maxillae with attached appliances were fixed with 10% of formaldehyde solution buffered with phosphoric acid for 5 days. Appliances were removed after the fixation.

The maxillae were decalcified with 4% formic acid for 5 days at room temperature, and embedded in paraffin wax. Blocks were sectioned into 6 μm thicknesses transversely or horizontally. All sections were stained with hematoxylin-eosin, Van-Gieson and PAS stain methods. The fibroblast nuclei were enumerated in a 125x125 μm^2 area delimited by an ocular grid.

Intermolar space between the first and second molars was measured by micrometer with an error of 0.1 mm division under the microscope (Figure 3). Fifty sections of each experimental and control group were prepared for nuclei counts and space measurement. Nuclei counts of experimental and control animals were compared by t-test.

Transmission electron microscopy

In the heavy force group, specimens were prepared for transmission electron microscopy during tooth movement as well as during retention (Table 1). The rats were fixed with a perfusion method by fixative solution containing 2.0% glutaraldehyde, 0.1mol/dm3 buffered cacodyle acid, and 7.0% saccharose, which had a pH value of 7.2. Gingival tissue between the first and second molars in the left side of maxillary arch, including transseptal fibers, was removed with a scalpel blade. Removed tissue was prefixed with previously described fixative solution for 90 minutes and then washed with 0.1 mol/dm3 buffered cacodyle acid containing 7.0% saccharose. Tissue was post-fixed with 1.0% osmic acid for 90 minutes. Fixed tissue was dehydrated with ethanol series, replaced with QY-1, and embedded in Epon 812. The block was sectioned with a diamond knife and stained with uranyl nitrate

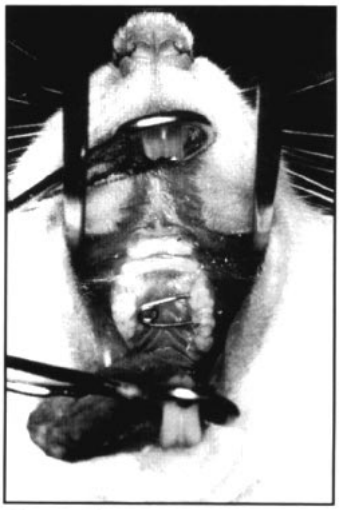

Figure 1

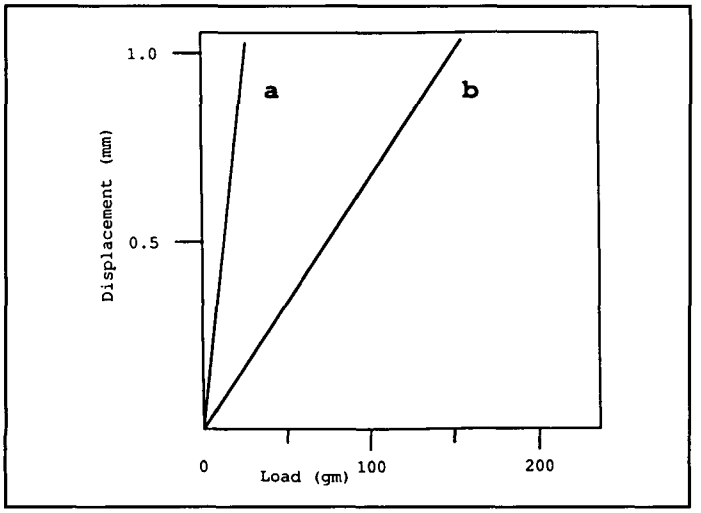

Figure 2

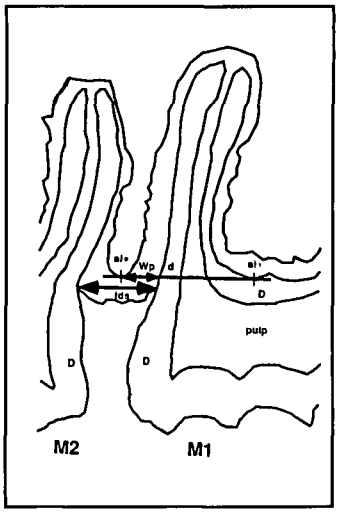

Figure 3

and lead citric acid. These sections were observed with transmission electron microscopy (Hitachi, model H-500, Tokyo, Japan).

Light microscopic autoradiography

The heavy force group at 1, 3 and 5 days was used for the study of light microscopic autoradiography (Table 1). 1.75μCi of L-[2-3H] Proline (Amersham, Tokyo, Japan), which had specific activity of 27.7 Ci/mmol/1 gm weight, was injected into the peritoneal cavity, and the animal was sacrificed 2 hours later. The maxilla was removed and fixed with 10% phosphate buffered formaldehyde for 5 days and decalcified with EDTA-2Na(pH7.2) at room temperature for 3 weeks. The block was embedded in paraffin wax and sectioned into 4 mm thicknesses. Sections were dipped in a half-concentration of Sakura autoradiographic emulsion NR-M2 (Sakura, Tokyo, Japan) for autoradiography. Dipped sections were exposed in a closed box at 4°C for 30 days. Silica gel was placed in the box as a drying agent. After exposure, each section was developed with Conidol X (Sakura, Tokyo, Japan) at 20°C for 5 minutes and fixed with Conifix (Sakura, Tokyo, Japan) at 20°C for 20 minutes. The sections were also stained with hematoxylin-eosin. The number of silver grains over the fibroblast and collagen fibers in a 50 x 50 mm^2 area was counted. The averages of 30 counts for three animals are presented with their standard errors (Table 3). Comparison of grain counts of experimental and control groups was made by a t-test.

Results

Intermolar distance between the first and second molars

The time-course of changes in the intermolar space between the first and second molars is shown in Figure 4. In the light force group, intermolar space increased by 0.2 mm at 1 day, and then continued to increase gradually to 0.8 mm at 10 days. In the heavy force group, intermolar space increased by 0.4 mm after 1 day, then ceased for 2 days, then gradually increased to 0.7 mm after 10 days. During mechanical retention periods, space was maintained at about 0.8 mm in both experimental groups.

Findings with light microscopy

1) Control group

Collagen fibers and fibroblasts ran wavy between cementum over alveolar bone. Each fiber was degenerated according to aging (Figure 5A-D).

2) Light force group during tooth movement

At 1 day, transseptal fibers were stretched mesiodistally according to tooth movement. Fibroblasts had elongated and assumed a more slender shape (Figure 6A). At 3 days, cell-free areas were observed in stretched fibers (Figure 6B). These areas were stained red with Van-Gieson staining and were positively stained with eosin and PAS. Five days after tooth movement, stretched fibers were more dense and fibroblasts were elongated. Part of these stretched fibers ran wavy at 10 days (Figure 6C).

3) Heavy force group during tooth movement

At 1 day, wide crevices were observed among the stretched collagen fibers, and the nuclei of fibroblasts adjacent to the crevices had fallen into pyknosis. The crevices were enclosed with the cell-free areas (Figure 7A). At 3 days, the number of collagen fibers and fibroblasts increased, and nuclei of fibroblasts adjacent to the cell-free areas appeared more oval (Figure 7B). At 5 and 10 days, the cell-free areas were replaced with dense collagen fibers and many fibroblasts.

Figure 1
Expansion spring for tooth movement.

Figure 2
Load-deflection curve of the springs. The expansion spring that was constructed with .012-inch round wire (a) exerted 25 gm by activation of 1.0 mm. The .018-inch round wire spring (b) exerted 150 gm by the same activation.

Figure 3
Tooth movement and periodontal membrane width.
Wp: width of the periodontal membrane; distance between alo and d;
alo: top of the alveolar crest between the first and second molars;
Ids: intermolar space;
al1: top of the alveolar septum of the first molar;
d: intersection of the line which connects alo and al1 with distal surface of the first molar;
D: dentin.
Distances were measured by micrometer with of an error of 0.1 mm, and were subtracted in the experimental groups from the control group as changes of periodontal width.

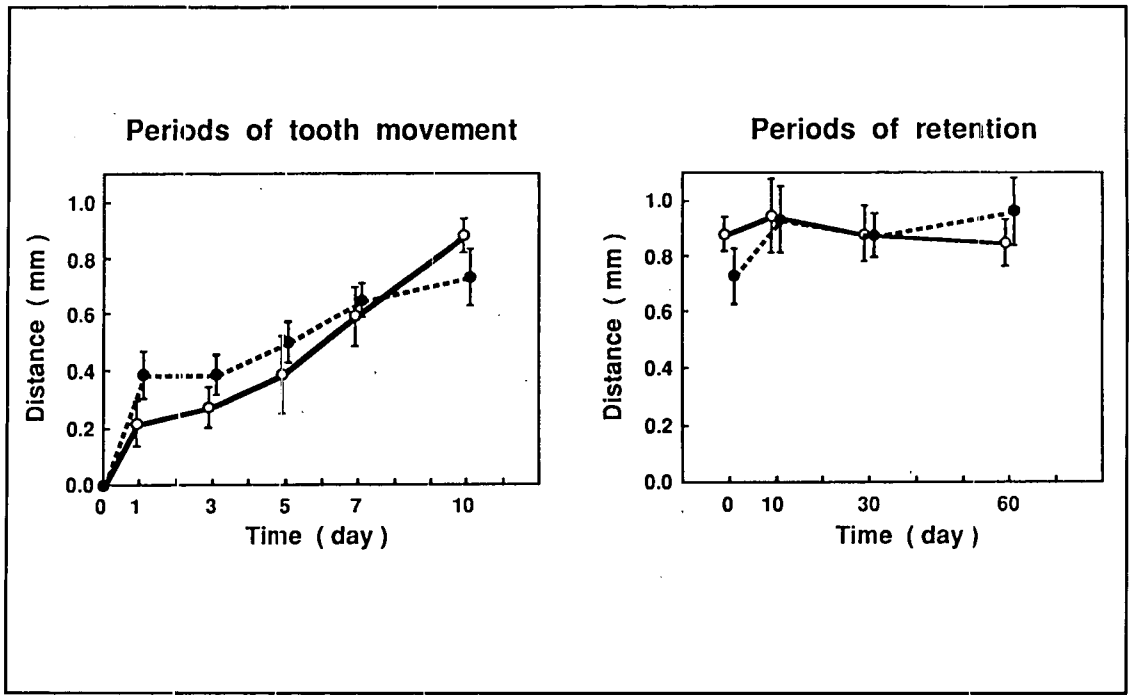

Figure 4
Time course of tooth movement.
O—O: increase of intermolar space in the light force group;
●—●: heavy force group;
After 1 day, intermolar space increased 0.2 mm in the light force group and 0.4 mm in the heavy force group. Minimal enlargement was observed for 2 days in the heavy force group, but after 10 days, space in both groups had increased to about 0.8 mm.

Figure 5A-D
Light micrographs of transseptal fibers in the control group.
A: control (1 day);
B: higher magnification of A;
C: control (60 days);
D: higher magnification of C (Van-Gieson's stain. Original magnification of A and C, x 4; B and D x 190).
Transseptal fibers lay over the alveolar crest between teeth. Each fiber became thinner and atrophied with age.

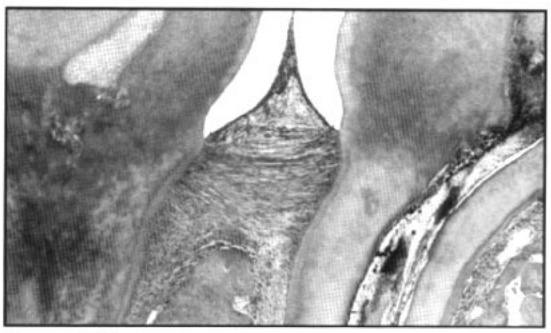
Figure 5A

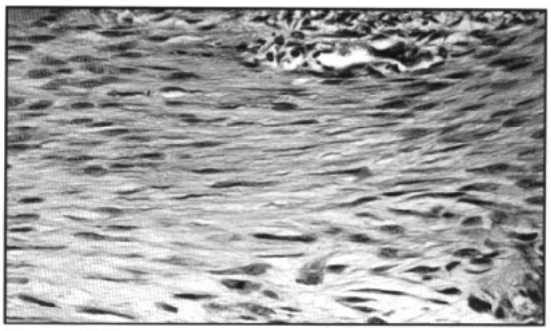
Figure 5B

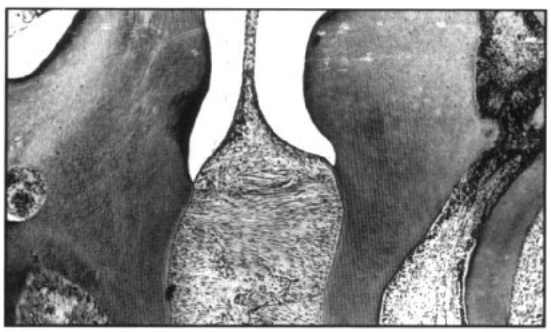
Figure 5C

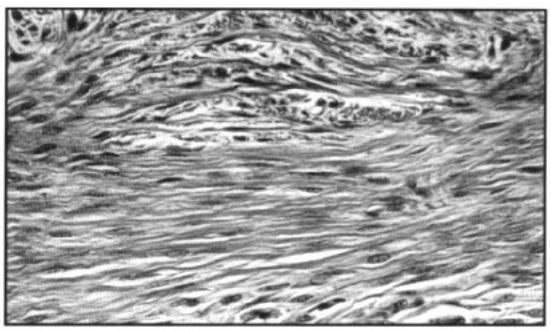
Figure 5D

Transseptal fibers were still stretched (Figure 7C).

4) Both groups during mechanical retention periods

In both experimental groups, no typical changes were observed in collagen fibers and fibroblasts after 10 days of mechanical retention. After 30 and 60 days of retention, transseptal fibers showed mild waving and became thicker (Figure 6D, Figure 7D).

Transmission electron microscopy

In general, fibroblasts were spindle-shaped, although some cells were flat. Nuclei assumed elongated, elliptical shapes, had smooth surfaces, and were unevenly distributed in the cytoplasm. Rough-surfaced endoplasmic reticulum (r-ER), mitochondria, Golgi-apparatus, and free ribosomes were observed in cytoplasm. After 3 days' tooth movement, fibroblasts, characterized by an abundant Golgi-region and r-ER, were promi-

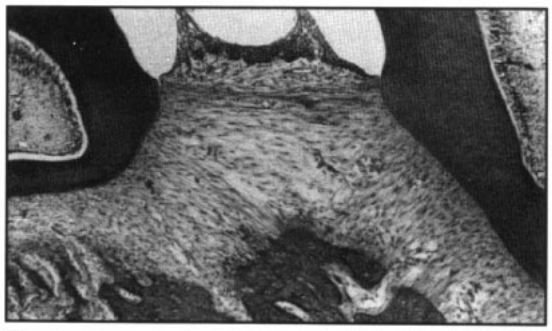

Figure 6A

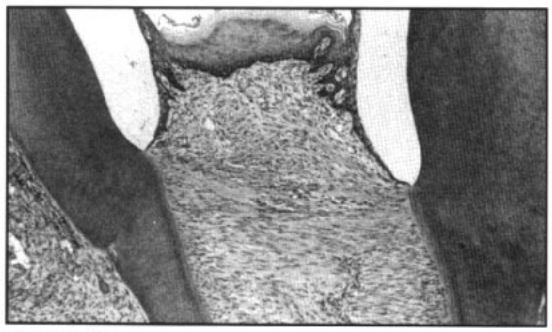

Figure 6B

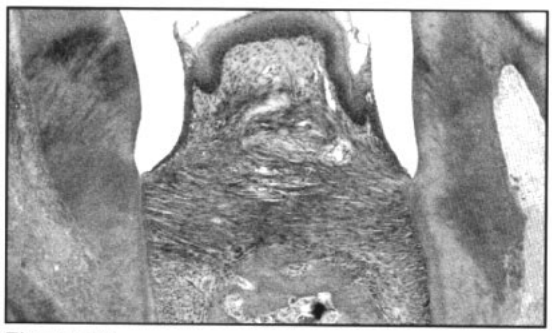

Figure 6C

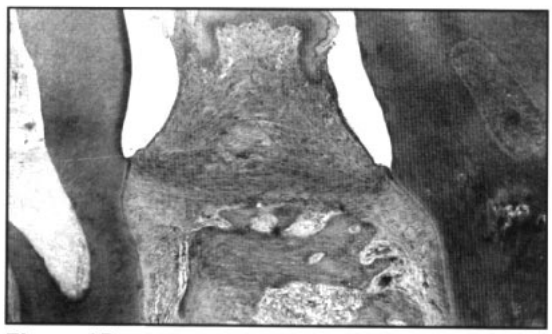

Figure 6D

Figure 6A-D
Light micrographs of transseptal fibers in the light force group.
A: 1 day after tooth movement;
B: 3 days after tooth movement;
C: 10 days after tooth movement;
D: retention for 60 days.
↑: semi-hyalinized area (Van-Gieson's staining, original magnification x 4). At 3 days, the cell-free area (semi-hyalinized area) appeared in the stretched fibers. However, it disappeared at 5 days.

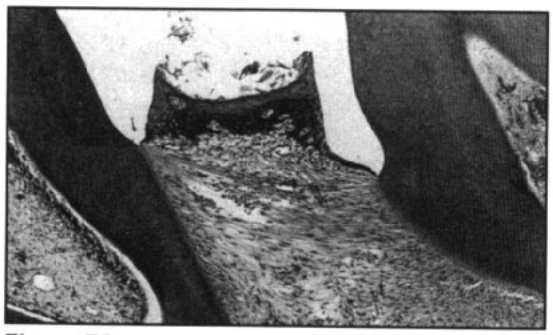

Figure 7A

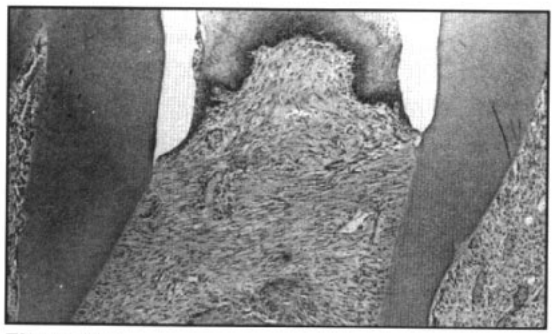

Figure 7B

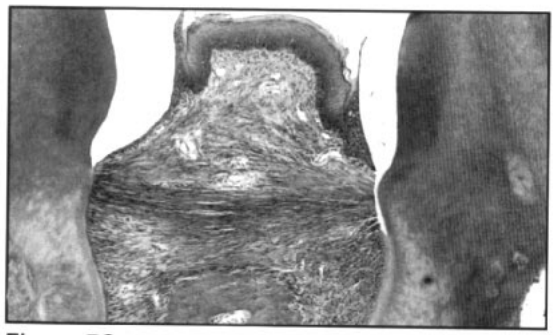

Figure 7C

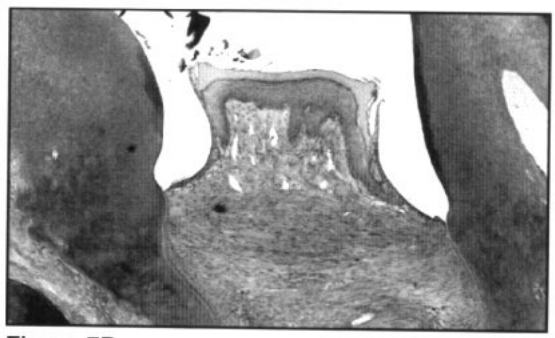

Figure 7D

Figure 7A-D
Light micrographs of transseptal fibers in the heavy force group.
A: 1 day after tooth movement;
B: 3 days after tooth movement;
C: 10 days after tooth movement;
D: retention for 60 days.
↑: semi-hyalinized area, ✳: crevices (Van Gieson's staining, original magnification x 4).
At 1 day, crevices were observed in the torn fibers and the semi-hyalinized area was observed. At 3 days, the semi-hyalinized area was enclosed with fibroblasts and at 10 days, it had disappeared and many fibroblasts were oriented in a mesiodistal direction.

nent. Among these cells were collagen-containing fibroblasts. These fibroblasts had some vacuoles that were covered with a smooth-walled limiting membrane that included banded collagen fibrils (Figure 8A). These vacuoles were observed alone or gathered in peripheral areas of the cytoplasm. In some cases, part of the vacuoles became large and contained electron-dense granular materials (Figure 8B). These two types of fibroblasts were also observed during mechanical retention periods.

Number of fibroblasts

The number of fibroblasts in a 125 x 125 μm^2 area during tooth movement and retention periods is shown in Table 2. The number of fibroblasts decreased significantly in the heavy force group, compared with the control group, at 1, 3 and 5 days, and decreased significantly in the light force group, compared with the control group, at 1 day. However, the number of fibro-

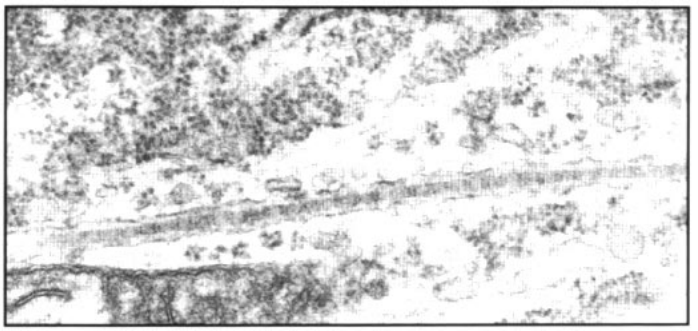

Figure 8A

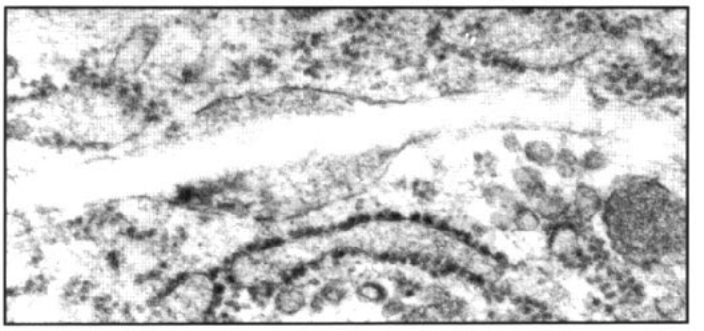

Figure 8B

Figure 8A-B
Electron micrograph showing the vacuoles containing collagen fibrils
A: Collagen fibrils were contained within the smooth-walled limiting membrane
B: Vacuoles containing banded collagen fibrils were spherical and contained electron-dense granular materials
↑:limiting membrane
***:electron dense granular materials (original magnification, A: x 4400 B: x 5100)**

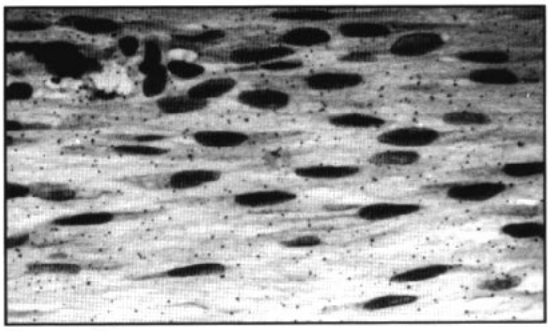

Figure 9A

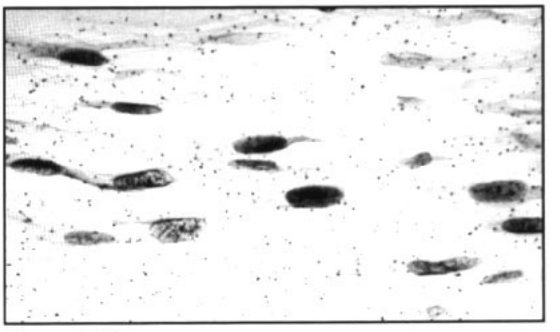

Figure 9B

Figure 9C

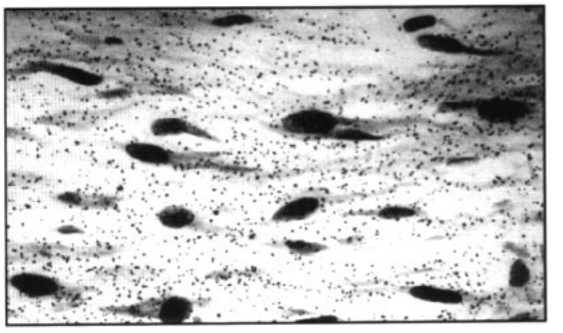

Figure 9D

Figure 9A-D
Light microscopic autoradiograph of transseptal fibers
A: control
B: 1 day after tooth movement
C: 3 days after tooth movement
D: 5 days after tooth movement (hematoxylineosin stain; original magnification x 380).

blasts recovered to the control level at 3 days in the light force group and at 10 days in the heavy force group. The number of fibroblasts in the control group decreased with age.

Number of silver grains in fibroblasts and collagen fibers

The time course of the number of silver grains (labeled proline) counted with light microscopic autoradiography is shown in Figure 9 and Table 3.

The number of grains in fibroblasts was significantly higher in the heavy force group than in the control group at 1, 3, and 5 days. There was no significant difference in the number of the grains in the collagen fibers between the control and the heavy force groups at 1 day. However, the number of grains increased significantly in the heavy force group when compared at 3 and 5 days.

Discussion

Macapanpan[3] and Edwards[13] reported on the initial changes of periodontal fibers during tooth movement. Woven collagen fibers in the periodontal membrane were stretched and reoriented to adapt to the increased distance between cementum and alveolar bone. However, collagen fibers in the periodontal membrane were broken down, and semi-hyalinized areas appeared when the distance increased further. These initial reactions were observed in transseptal fibers of this study and were dependent on the degree of force applied to the teeth. Semi-hyalinized areas were consistent with a degenerative process.[19-21] In the periodontal membrane, orthodontic force caused the disturbance of blood supply,[22-28] and tissue activity decreased according to the amount of stress. As a result, the number of fibroblasts decreased and periodontal fibers fell into semi-hyalinization (cell-free area) or hyalinization. Reitan[19] and Okumura[21] stated that the hyaliniza-

Table 2
Changes in number of fibroblasts

	During tooth movement					After tooth movement		
	1 day	3 days	5 days	7 days	10 days	10 days	30 days	60 days
Control group	35.9±3.3	36.5±4.3	35.5±3.9	33.9±3.9	33.9±3.9	33.5±4.3	32.7±4.7	31.8±3.8
Light force	31.2±5.5**	36.9±4.7	36.9±4.7	33.5±4.3	33.8±4.4	34.5±3.0	34.5±3.2	33.2±3.7
Heavy force	19.7±8.9**	26.6±6.1**	32.4±4.5*	32.7±5.5	31.9±5.2	33.9±4.5	33.1±3.3	32.9±3.2

Results expressed as mean ± S.D.

* P<0.025, ** P<0.01

Table 3
Number of grains in collagen fibers and fibroblast

		Tooth movement		
		1 day	3 days	5 days
Collagen fibers (50 μm x 50 μm)	control group	106.8±22.1	96.4±13.2	100.3±14.5
	heavy force group	104.6±31.4	200.2±56.1**	212.4±45.6**
		(97.9)	(207.7)	(211.7)
Fibroblasts	control	4.5±1.0	5.2±0.8	5.2±1.0
	heavy force group	7.2±2.9**	7.7±2.8**	7.5±2.3**
		(160.0)	(148.1)	(144.2)

Results expressed as mean ± S.D.
Parentheses: percentage to the control, **P<0.01

tion was resolved as a foreign body by macrophages or osteoclasts and replaced with inflammatory tissue; the semi-hyalinization was possibly a reversible reaction. In transseptal fibers in this study, the number of fibroblasts decreased and the semi-hyalinized area was observed for 3 days in the heavy force group and for 1 day in the light force group. The semi-hyalinized area was enclosed with fibroblasts. Under electron microscopic observation, some vacuoles containing banded collagen fibers in the cytoplasm could be seen in the fibroblasts adjacent to the semi-hyalinized area. This finding suggests that fibroblasts have a role in absorbing collagen fibers. Other investigators[29-32] have reported collagen phagocytosis or resorption within fibroblasts.

On the other hand, synthesis and turnover of the collagen fibers has been studied with autoradiography.[33,34] Cho[33] reported on the synthesis of collagen fibers in the periodontal membrane; ^{3}H proline autoradiographic grains were observed over fibroblasts 3 minutes after injection and found over extracellular collagen fibers 20 minutes after injection. Turnover time of periodontal and gingival tissue has been analyzed by autoradiography. Rippin[17,18] reported that half-lives of the incorporated proline was 4.8 days in periodontal tissue of the mouse. However, Orlowski[34] stated that the half-life of the activity was approximately 24 days in gingival tissue. Turnover time seemed to be slower in gingival tissue than in periodontal tissue.

Moreover, changes in the turnover time in periodontal and gingival tissue due to tooth movement were studied. Boisson[5] reported that increased incorporation of ^{3}H proline continued in periodontal and transseptal fibers until 14 days when tooth movement lasted for 14 days, and that incorporation of ^{3}H proline recovered to the control level after tooth movement. Matuura[29] showed the incorporation of ^{3}H proline in fibroblasts of periodontal tissue. He re-

ported that the number of grains increased for 10 days according to tooth movement. Crumley[4] reported on periodontal reaction to tooth movement and indicated that the number of cellular grains was higher in the experimental group than in the control; however, it gradually decreased and there was no difference in the number of grains between experimental and control groups at 3 days. Furthermore, he reported that the number of extracellular grains increased for 12 hours and gradually decreased.

In this study, the tissue reaction to orthodontic stimuli was noticeable for 1, 3, and 5 days in the heavy force group under microscopic observation. Intermolar space was dominantly enlarged for these same periods. Autoradiographic study was planned to assess the synthetic response to an increased intermolar space. The grains over fibroblasts had already increased to 150% at 1 day, and the grains over collagen fibers increased to 200% at 3 days. These reactions indicate that orthodontic stimulation activates collagen synthesis of fibroblasts in transseptal fibers and maintains it at high levels during tooth movement.

These studies suggest that the synthetic activity of periodontal fibers is increased by the application of force, and the rate of increase is higher in periodontal membrane than in transseptal fibers. However, it is not certain whether turnover time of collagen is prolonged or shortened after activation of collagen synthesis. Rippin[17,18] reported that turnover time showed no change in the hyper function of periodontal membrane and hypothesized that functional stress within physiological limits was not important in determining turnover rates.

In our study, changes of the width of the periodontal space at the alveolar crest and changes in the intermolar space were measured. Histological and autoradiographic study showed that collagen synthesis activity in the transseptal fibers is depressed for a definite time by the initial tooth movement and reversibly recovers to the control level in 3 to 5 days. Intermolar space is increased by 0.4 mm at 1 day and plateaus for 2 days. The major changes in the number of fibroblasts and incorporated grains appeared in 3 days. These results suggest that the transseptal fibers were remodeled early with the proliferative response of cell numbers and the collagen synthesis of fibroblasts.

If orthodontists control this proliferative response of fibroblasts, retention periods for moved teeth will be shortened.

Iida[22] reported that vascular permeability in the periodontal membrane increased at 3 and 12 hours, and Yamaguchi et al.[28] reported that vas-

cular permeability in the palatal soft tissue increased at 10 hours. Saito et al.[36] reported that prostaglandin and cytokine interleukin-1 were localized immunohistochemically in stressed periodontal ligament, and that these cytokines affect bone resorption during tooth movement. But, in the transseptal fibers, no cytokine that might affect the remodeling mechanism was reported. We are preparing for the simple model of transseptal fibers in vitro by human gingival fibroblasts to analyze the cytokines that regulate the remodeling mechanisms.

Further investigation of the reaction of the transseptal fibers caused by tooth movement is indicated.

Conclusion

The remodeling mechanisms of transseptal fibers during tooth movement and retention periods were investigated. Morphological characteristics of collagen fibers and fibroblasts were studied histologically. Transseptal fibers were rearranged early during tooth movement and gradually remodeled during retention periods; fibroblasts were employed in the remodeling by proliferation as well as in the synthesizing and degrading of collagen fibers.

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