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SCIENTIFIC SECTION

Experimental tooth movement under light orthodontic forces: rates of tooth movement and changes of the periodontium

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Abstract

Aim: To investigate light forces for experimental tooth movement.

Method: Light orthodontic forces of 1.2, 3.6, 6.5, and 10 *g* force (gf) were applied for 14 days to move rat molars, and the effects of the forces on the rate of tooth movement and changes of the periodontium were examined.

Results: In the early period, despite the different levels of force used in each group, there were no significant differences in tooth displacement. From hour 56 to day 14, the tooth displacement in the 1.2 gf group was significantly smaller than that in the other groups and the rate was nearly constant. The rates of tooth displacement in the 3.6, 6.5, and 10 gf groups fluctuated repeatedly, while the orthodontic forces gradually decreased.

Conclusion: Experimental tooth movement in rats, tipping without friction under light forces, were either constant or fluctuated in cycles of several days' duration. This is in contradiction to the three-phases-theory of tooth movement described in previous investigations using heavy forces.

Index words: Experimental tooth movement, light orthodontic force, rate of tooth movement, titanium-nickel alloy wire.

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Introduction

Since Sandstedt first reported the histological changes of the periodontium during orthodontic tooth movement, this subject has been discussed by many authors[1–3]. In animal studies, more than 20 gf have been used to move rat molars[4–9]. These forces correspond to more than 400 gf for the human first molar, after correction for differences in root area.[10]

Other studies have investigated the periodontal ligament (PDL) from the viewpoint of activity in the microvasculature, and have reported optimum orthodontic pressures of 20–25 g/cm[2,11] or under 80 g/cm[2,12]. Based on these previous researches, the optimum force for the bodily movement of the rat molar is less than 10 gf. However, the rate of tooth movement and the changes of the periodontium under such light orthodontic forces have not been discussed.

Accordingly, in this study, we examined the rate of tooth movement and the changes of the periodontium under light orthodontic forces of less than 10 gf.

Materials and methods

Animals

Forty male Sprague–Dawley (SD) strain rats were used in this study. All animals were fed on powder form fodder (Rodent Diet CE-2; Japan Clea Inc., Shizuoka, Japan) and drinking water *ad libitum*. In addition to orthodontic forces, occlusal force,[13–15] and cheek and tongue pressure[16–18] may act on teeth during experimental tooth

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movement. In this study, all of the mandibular left molars were extracted at 4 weeks of age in order to eliminate the influence of occlusal forces caused by occlusal contact with the opposing side (Figure 1a). The rats were intraperitoneally anaesthetized with ketamine hydrochloride (Ketalar 50; Sankyo Co, Ltd., Tokyo, Japan) containing 20 per cent xylazine hydrochloride (Celactal 2 per cent injections; Bayer-Japan Co, Ltd., Tokyo, Japan) as a muscle relaxant, after anaesthetization by inhaling diethyl ether. To assess whole body effects the animals were weighed before each treatment. All procedures followed the guidelines of the Tokyo Medical and Dental University for Animal Research.

Procedures of tooth movement

The tooth movements were started when rats were 5 weeks of age. Forces were applied to the maxillary left first (M1) and second (M2) molars in the mesio-distal direction reciprocally for 14 days[19] (Figure 1b). Bucco-palatal enamel grooves 3.2 mm apart were cut in the occlusal surface of M1 and M2 with a steel bar (plate saw; Maillfer, Ballaigues, Switzerland) under cooling with an air spray. The enamel around the grooves was etched with 65 per cent phosphoric acid for 30 seconds. Both ends of an orthodontic wire were set into the groove and attached with light cure resin (Clearfil Photo SC; Kuraray Co. Ltd., Osaka, Japan).

Work-hardened titanium-nickel alloy (Ti-Ni) wires, 0.152 mm in diameter and 12 mm in length (Furukawa Electric Co. Ltd., Tokyo, Japan) were used to exert the orthodontic forces. The wires were bent so as to exert initial forces of 1.2, 3.6, 6.5, and 10 gf when the distance of the both ends was 3.2 mm. The initial force was measured by load cell (FGX-0.2 digital force gauge; Shimpo, Kyoto, Japan).

The load produced as a result of displacement was measured with the load cell by simulating 14 days of tooth displacement (Figure 2). Rats of the same age were used for the sham operations; their mandibular left three molars were extracted, and the grooves in the occlusal surface of the M1 and M2 were cut and filled with the same resin, but without wire fixation.

Examination of the time course of tooth displacement

During the 14-day experimental period, laminated hydrocolloid (Dentloid super green; Dentronix, Tokyo, Japan) and alginate impressions (Algiace 2; Sankin Co. Ltd., Tokyo, Japan) were taken three times a week (every 56 hours) and used to make dental stone (New plastone; G.C. Co. Ltd., Tokyo, Japan) models of the maxillary left molar.[19] These impression materials and stone models could reproduce a line of 50 μm in width.[20]

The inter-proximal space between M1 and M2 was measured three times for each object using a non-contact digital microscopic gauge (MS-214; FUSOH Co. Ltd, Tokyo, Japan) with a minimum readable distance of 10 μm, and the mean value was taken as the distance of tooth displacement D_i [μm] ($i = 0, 1, \ldots, 6$).

Before the measurement, we investigated the margin of error for individual experimenters, as well as the margin of error among experimenters. They were analysed with analysis of variance (ANOVA) followed by Scheffe's *post hoc* test using Stat View 5.0 soft ware (SAS Institute, Cary, NC). No significant differences were found among the three measurements of any of the experimenters or among the collective results for the five experimenters ($P > 0.05$).

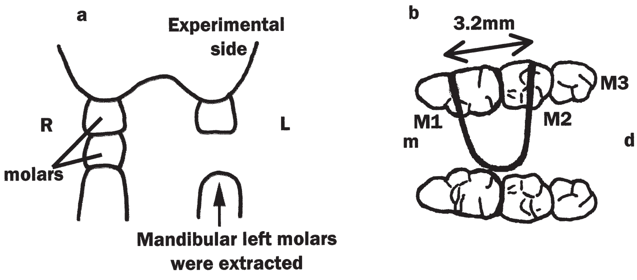

Fig. 1 Experimental model. (a) A schema of frontal section in rat molar. All of the mandibular left molars were extracted in order to eliminate the influence of occlusal forces caused by occlusal contact with the opposing side. (b) Maxillary occlusal surface. Both ends of the orthodontic wire were set into the bucco-palatal enamel groove and attached with light cure resin. m: mesial, d: distal, L: left, R: right.

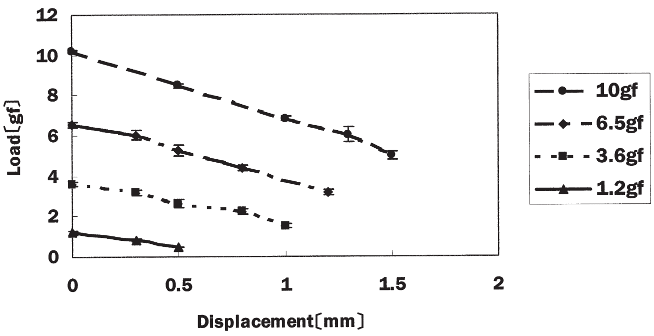

Fig. 2 Load-displacement curves (mean ± SD). The load diminished slightly and almost linearly as the tooth displacements increased.

The experimental period was divided into 6 prescribed periods of 56 hours, and the rates of tooth displacements per hour V_i [μm/hour] ($i = 1, 2, \ldots, 6$) were worked out from the distance of tooth displacement each period as follows:

$$V_i = (D_i - D_{i-1})/56$$

In order to examine the fluctuation in the rate of tooth displacement for each period, the changes of the rate of tooth displacement ΔV_i [μm/hour] ($i = 1, 2, \ldots, 5$) were determined as follows:

$$\Delta V_i = V_{i+1} - V_i$$

Histological examination

On days 7 and 14 of the tooth displacement, animals were anaesthetized with diethyl ether and killed by dislocating their cervical spines. The maxillary specimens were immediately removed, fixed in a solution of 4 per cent paraformaldehyde and 0.1 M cacodylic acid buffer (pH 7.3) at 4°C for 18 hours, decalcified in 10 per cent EDTA solution at 4°C for 30 days, and embedded in paraffin by a conventional method. Five-μm thick serial sections of the roots of the M1 and M2 were cross-sectioned with the surrounding tissues with a microtome, and stained with haematoxylin and eosin.

The distobuccal root of M1 was selected for observation because preliminary experiments showed that the distobuccal root was positioned vertical to the occlusal surface and had less deformity or physiological root resorption. In addition, due to the tipping effect—which is most obvious in the furcation area[17]—an area was chosen 200 μm away from the crest of the furcation area toward the root apex.

Statistical analysis

The tooth displacement among each group was analysed by ANOVA followed by Scheffe's *post hoc* test using StatView 5.0 software (SAS Institute, Cary, NC). A level of $P < 0.05$ was considered to indicate statistical significance. Because the rate of the tooth displacement in each animal varied, a Mann–Whitney *U*-test was used to analyse the standard deviation of changes of the rates of tooth displacement for each animal every 56 hours. This indicates the alteration of the rates of tooth displacement during experimental period and larger value reveals higher deviation of the rates of tooth displacement.

Results

Weight change of animals

The average weights of the rats increased continuously in both the experimental and control groups. No significant difference in weight was found among groups (Figure 3).

Macro-findings of tooth displacement

Three weeks after the extraction of mandibular molars, the inter-proximal area between M1 and M2 of the sham-treated controls, was in contact with no remarkable elongation (Figure 4a). After 14 days of tooth movement, mesial inclination of the M1 and distal inclination of the M2 were evident in the buccal view (Figure 4b,c).

Aspects of tooth displacement

Changes of distance of tooth displacement in each group. At the first 56 hour measurement, the average tooth displacement among the four groups was 0.2 mm, and there were no significant differences in tooth displacement among groups (Figure 5). From the second 56 hour measurement through day 14, the displacement was increased, but that of the group receiving 1.2 gf was significantly smaller than that of the other groups ($P < 0.05$). Although there was no significant difference, the groups receiving larger initial force tended to show greater tooth displacement than those receiving lower initial force. The orthodontic forces during the experimental period diminished slightly and almost linearly as the tooth displacement increased (Figure 2).

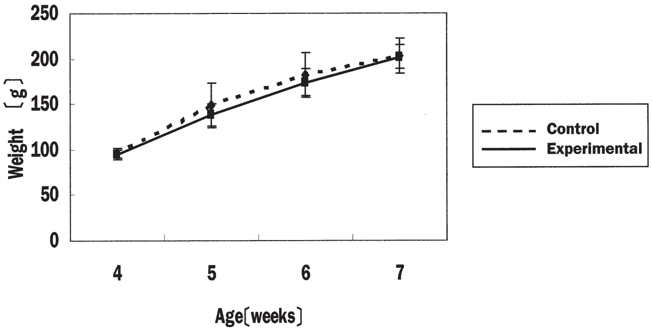

Fig. 3 Changes in body weight (mean ± SD). The weights of the rats increased continuously in both groups with no significant difference in weight between groups.

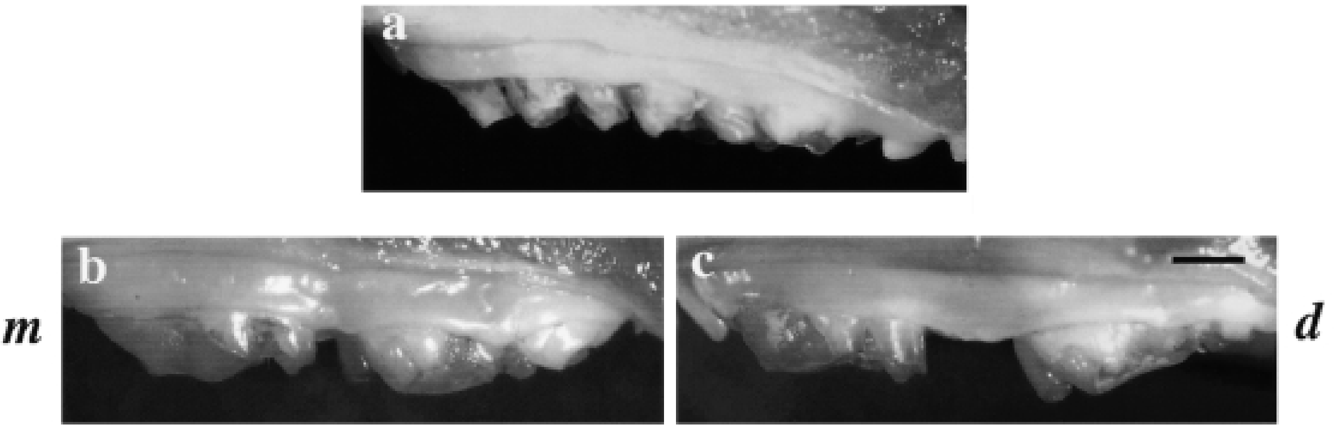

Fig. 4 Macro-findings of tooth displacement on days 14 (3 weeks after the extraction of mandibular molars) at buccal view (bar: 1 mm). (a) Control group. (b) Initial force 1.2 gf. (c) Initial force 10 gf. m: mesial side; d: distal side. Control group had close contact and no remarkable elongation was observed (a). Mesial inclination of the M1 and distal inclination of the M2 were evident (b,c).

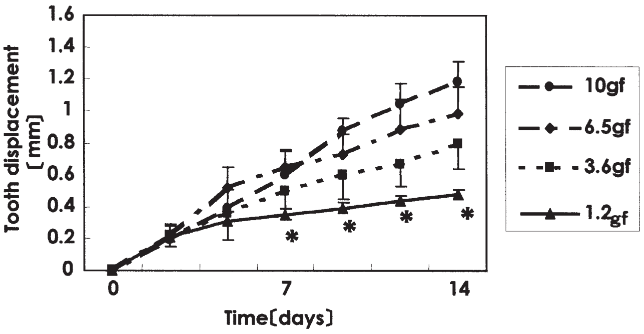

Fig. 5 The tooth displacement (mean ± SD). The tooth displacement gradually increased through 14 days. At the first 56-hour measurement, there were no significant differences in tooth displacement among groups. In the following periods, the displacement of the 1.2 gf group was significantly smaller than that of the other groups ($*P < 0.05$).

The rate of tooth displacement in each rat.

At the 56 hours observation point, the rate of tooth displacement in 1.2 gf group changed little and the movement was almost constant after the initial 56 hours period (Figure 6a). On the other hand, the teeth in the other groups moved quickly and slowly in repeated cycles every few days (Figure 6b–d).

The deviation of changes of the rates of tooth displacement for each animal at every 56 hours are shown in Figure 7. The 3.6 and 10 gf group were significantly larger than the 1.2 gf group ($P < 0.05$). In other words, these data indicated that the rates of tooth displacement in 3.6 and 10 gf groups fluctuated more than 1.2 gf group.

The standard deviation of the changes of the rates of tooth displacement in the 6.5 gf group were not significantly different compared to the 1.2 gf group, however, the changes in the rates of tooth displacement also tended to fluctuate in every 56 hours in this group (Figure 7).

Change of the periodontium

On days 7 and 14 in the groups receiving 1.2 and 3.6 gf force, the widths of the PDL were slightly narrow, but no hyalinized tissue was evident. Multinucleated cells, which were considered to be osteoclasts, were seen on the surface of the alveolar bone and direct bone resorption was observed subjacent to these cells (Figure 8b,c,f,g).

A narrow PDL, limited hyalinization and frontal bone resorption were all evident on days 7 and 14 in the 6.5 gf group (Figure 8d,h). At day 7 in the 10 gf group, the root touched the alveolar bone and hyalinization with local undermining bone resorption was observed in the surrounding area (Figure 8e). On day 14 in the 10 gf group, however, only a very limited area of hyalinization was seen, and direct bone resorption took place rather than undermining bone resorption (Figure 8i). The root resorption seen in these experiments was mainly in the cementum lacunae. The largest lacunae were found in the 10 gf group on day 14 (Figure 8i), but no severe resorptions, which were observed with forces of higher magnitude, were seen.

Discussion

In spite of the different forces used in each group, the tooth displacements were on average 0.2 mm without

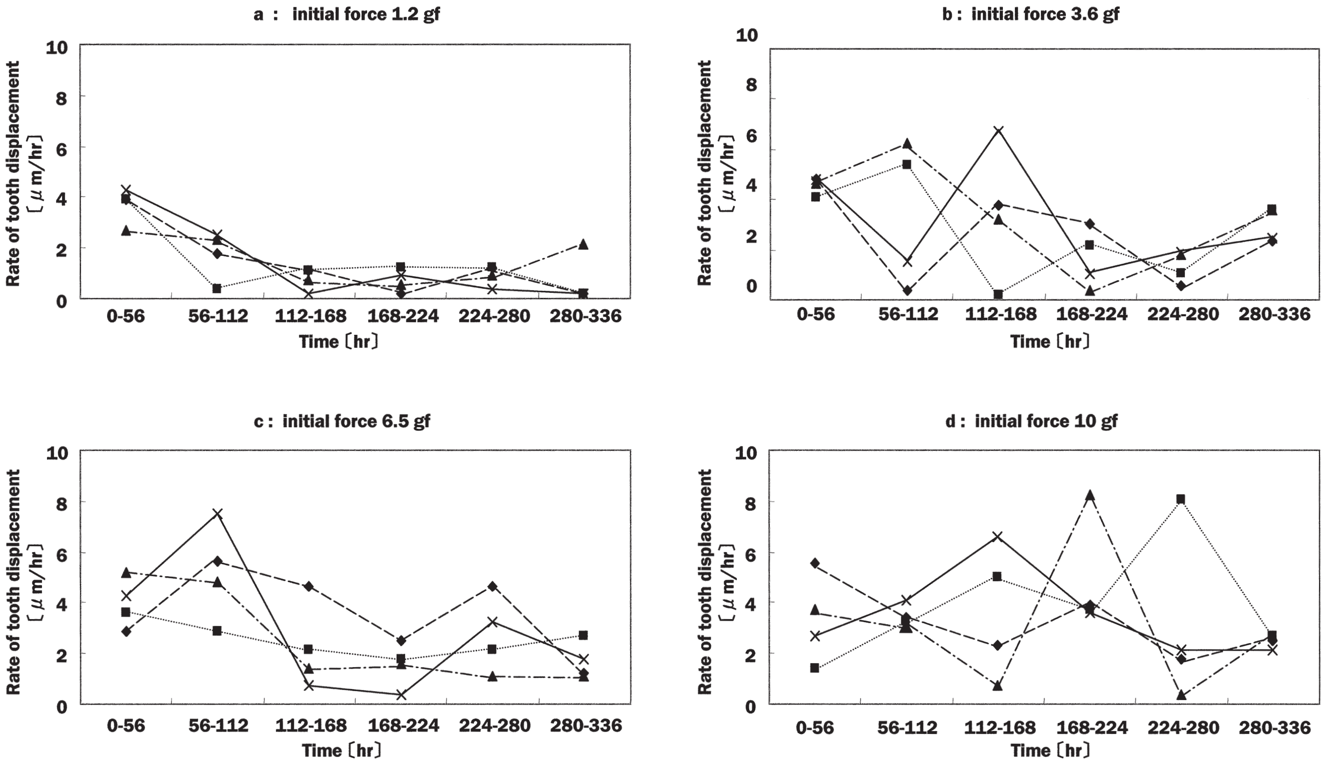

Fig. 6 The rate of tooth displacements in each rat. In the 1.2 gf group, the rate of tooth displacement changed little and the movement was almost constant after initial period (a). The teeth in the other groups moved quickly and slowly in repeated cycles every few days (b–d).

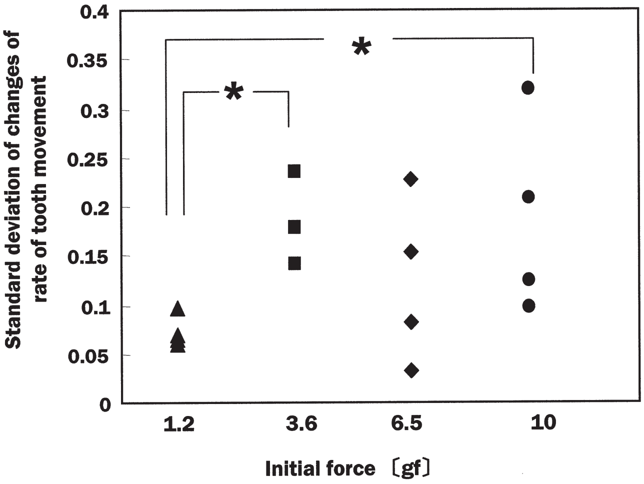

Fig. 7 The standard deviation of change of the rate of tooth displacement in each animal. The vertical line indicates the alteration of the rate of tooth displacement during experimental period and larger value reveals higher deviation of the rate of tooth displacement. The rate of tooth displacement in 3.6 and 10 gf group, fluctuated more than in the 1.2 gf group ($*P < 0.05$). The 6.5 gf group was not significantly difference compared to 1.2 gf groups, however, the changes in the rate of tooth displacement were more various, and the rate of tooth displacement in this group also tended to fluctuate every 56 hours.

any significant differences among the four groups during the first 56 hours. Several authors have reported a PDL of 0.1 mm width in normally occluded rats.[21, 22] Since the type of tooth movement in our study was tipping the tooth displacement of 0.2 mm in the first 56 hours might be explained by the visco-elastic modification of PDL and the strain of the alveolar bone.[23, 24]

In the 1.2 gf group, the rate of tooth displacement was fastest in the first 56 hours, which then decreased, in a gradual, constant fashion to day 14. The slight changes in the rate of tooth displacement at each 56-hour measurement also suggest even tooth movement in this study group. These data suggest that there were two phases of tooth movement in this experimental group: an initial shifting of the tooth due to the compression of PDL, followed by a smoother rate of movement for the rest of the experimental period. In the histological views on days 7 and 14, direct bone resorption with osteoclasts on the surface of the alveolar bone in the pressure side was observed without any hyalinization or undermining bone resorption.

It is generally considered that tooth movement due to heavy orthodontic forces can be divided into three

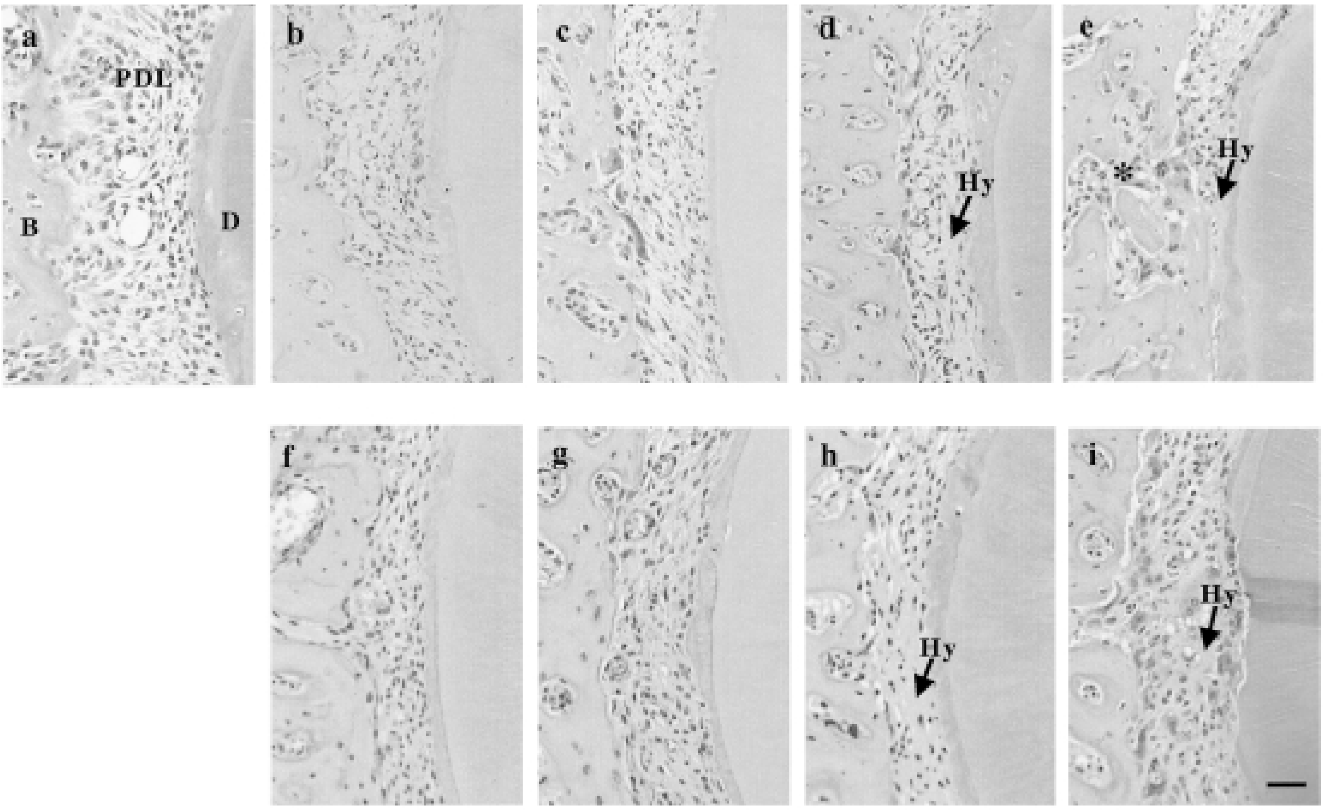

Fig. 8 Mesial side of the distobuccal root in maxillary M1 (H&E stain). These regions were 200 μm away from the crest of the furcation areas toward the root apex (bar: 50 μm). (a) control (3 weeks after the extraction of mandibular molars). (b–e) On day 7. (f–i) On day 14. B: alveolar bone; D: dentin; PDL: periodontal ligament; Hy: hyalinized tissue; *undermining bone resorption. On days 7 and 14 in the groups receiving 1.2 and 3.6 gf force, the widths of the PDL were narrowed slightly, and no hyalinized tissue was evident. Multinucleated cells, which were considered to be osteoclasts, could be seen on the surface of the alveolar bone, and direct bone resorption under these cells was observed (b,c,f,g). Narrow PDL, limited area of hyalinization, and direct bone resorption were all evident on days 7 and 14 in the 6.5-gf group (d,h). At day 7 in the 10-gf group, the root touched the alveolar bone, and limited hyalinization with local undermining bone resorption was observed in the surrounding area (e). On day 14 in the 10-gf group, only a very limited area of hyalinization was recognized and direct bone resorption took place (i). Minor root resorption in the cementum was observed in some regions (i).

phases: an initial strain consisting of the changes in the visco-elasticity of PDL and the distraction of alveolar bone with a few days, followed by a lag phase in which the tooth movement slows down for 1 or 2 weeks with the hyalinization in the PDL and finally a phase in which the tooth moves progressively with evident undermining bone resorption.[3,7,24–26] Our findings suggest that the tooth movement induced by light forces with modern clinical appliances are close to physiological movements. They are clearly different movements from those seen with heavy forces.

Neither the 1.2 gf nor the other groups showed tooth-movement features similar to those in the traditional three-phases-theory in which the second phase consists of a slowing or cessation of tooth movement for 1–2 weeks. The data in Figures 6 and 7 suggest that the rate of tooth displacement in the 3.6, 6.5, and 10 gf groups fluctuated in cycles of several days' duration. Such fluctuating rates of tooth displacement seemed to be independent of the gradually dissipating orthodontic forces, which indicated that the magnitude of force is not the only component determining the rate of tooth displacement. From a histological viewpoint, the widespread hyalinization and undermining bone resorption that are the features of the second phase of traditional theory were not evident even in these groups. The teeth in these groups might move, while alternating between the two phases, that is, the phase in which the PDL is compressed and the roots are adjacent to alveolar bone, and the phase where direct bone resorption recovers the width of PDL. Our investigation and former reports suggest that the compression of the PDL, the existence of hyalinized tissue, as well as direct or undermining bone resorption on the pressure side may regulate the rate of tooth displacement. Collagen fibres,[27] oxytalan fibres,[28] and alveolar bone remodelling on the tension side may also limit the rate of tooth displacement.

In conclusion, the experimental tooth movements in rats using light orthodontic forces and tipping without friction were almost constant or fluctuated over a cycle of several days' duration. This contradicts the three phases theory of tooth movement in previous investigations using heavier forces.

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