รายงานวิจัย

การศึกษาระยะที่ 1

เรื่อง

การทดลองเคลื่อนฟันในหนู โดยเครื่องมือจัดฟันชนิดใสอย่างเป็นลำดับ

(Experimental tooth movement by sequential orthodontic appliances in rats)

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จากงบประมาณเงินรายได้มหาวิทยาลัยศรีนครินทรวิโรฒ

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Experimental tooth movement
by sequential orthodontic appliances in rats

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Abstract: Sequential invisible appliances have been recently introduced to orthodontic treatment. Evaluation on the biomechanics and biology of tooth movement in response to orthodontic forces exerted by these appliances is still necessary. **Objectives:** This preliminary study aimed to describe early histological changes of paradental tissues in response to a clear plastic appliance and to evaluate tooth movement by using radiographs as compared with a closed-coil spring in rats. **Methods:** Fifteen male, 40-50-day-old Sprague-Dawley rats were randomly divided into 3 groups. Implant markers were placed on the palates prior to the experiment. Group I served as untreated controls. Rats in Group II received a plastic appliance made from a model of which the maxillary left first molar was set up mesially 0.5 mm. from the original position. The appliance was bonded to the maxillary teeth on the contra-lateral quadrant and incisors. Group III had a fixed appliance to move the maxillary left first molars mesially by a closed-coil spring. Lateral radiographs and occlusal films were taken at initial, day 1, day 4, and day 7. Specimens were prepared in parasagittal sections in order to investigate early histological changes of paradental tissues of mesio-buccal and distal roots of the maxillary left first molars in all groups by a light microscope. **Results:** In Group II after day 1, the periodontal ligament (PDL) was compressed in the bifurcation and apical area of the roots of the maxillary left first molar. By day 7, PDL of apical and distal aspects of the roots and bifurcation presented with further compression whereby stretched PDL of mesio-buccal root along the mesial side was noticed. In Group III, disorganized and compressed PDL in mesial cervical half and apical area of the roots as well as inter-radicular septum was observed from day 1 whereas the stretched fibers presented at distal aspects of the roots after day 4 to 7. Superficial cementum resorption could be seen in areas of compression from day 1 in both groups. From radiographs, there was no significant difference between the groups from day 1 to day 7 in incisal to palatal plane angle and distance from the incisors and molar to the implant markers. **Conclusions:** The results suggested that early histo-biomechanics of tooth movement in response to the clear plastic appliance may be intrusion and distal tipping despite the intended mesial movement.

Key Words: orthodontic, plastic, rat, sequential

INTRODUCTION

Sequential removable orthodontic appliances have been recently introduced to clinical orthodontic practice using an advanced, adjunctive computerized technology.[1-3] This technique (Invisalign, Align Technology, Inc., Santa Clara, California) gives an opportunity for dental practitioners to plan for sequences of tooth movement from initial to final position by using computerized graphical images of patients' dental models.[4] Sequential removable clear plastic appliances are then fabricated from stereolithography models of these images. Recent clinical trials demonstrated no statistical difference in ability to complete the treatment in term of types of material (soft versus hard clear plastic)[5] and indicated that anterior alignment was the most improve component of the weighted PAR score whereas the buccal occlusion was the least improved.[6] Though there are case reports of successful treatment using these appliances, further research studies are needed to investigated. Frequently, daily orthodontic practice is approached by clinical experiences rather than enough evidence based knowledge. Basic biomechanics of tooth movement and histological changes of the paradental tissues after force application by these sequential invisible appliances is still necessary to evaluate. The planned sequential tooth movement by these removable plastic appliances is not yet explained in terms of applied force system and tissue reactions describing by bone remodeling and cellular responses.

Experimental animal models for studies of orthodontic tooth movement are performed to achieve more profound information of biological response to orthodontic forces. Despite questions of findings from animal models can be extrapolated to human beings, experiments in animals especially rats are generally considered to be a good model for studies of orthodontic tooth movement.[7] The design of force delivery system used in the rat model is often a closed-coil spring fixed to the molar or molars and the incisors to produce a continuous force.[8-13] This resulted in mesial tipping movement of the molars. Reitan's reports confirmed the findings that, during orthodontic tooth movement, the periodontal ligament (PDL) is compressed in areas where tooth is move toward the alveolar bone in conjunction with alveolar bone resorption so that the tooth is relocated.[14, 15] He also demonstrated stretched PDL presents in zones where the tooth is moved away from the bone. These findings of histological studies were extrapolated to describe types of biomechanics of tooth movement, i.e. tipping or bodily movement, which later may be defined as histo-biomechanics of tooth movement. It would be advantageous to apply the known designed experiments in rats and compare to this clear plastic orthodontic appliance in order to identify its histological tissue reaction.

The purposes of the study were to describe early histological tissue responses after force application by a clear plastic orthodontic appliance in rats and to determine initial biomechanics of tooth movement by using radiographs as compared to force application by a conventional coil spring.

MATERIALS AND METHODS

Animals and experimental procedures

The study comprised fifteen male, 40-50-day-old Sprague-Dawley rats weighing 287±38 g. All animals were housed under the standard guideline of the National Laboratory Animal Center, and they were fed a standard pellet diet (No. 082, National Laboratory Animal Center, Thailand) with tap water *ad libitum*. The weight of the animals was recorded on the day of appliance insertion and before death. The experimental protocol was approved by the Committee for Animal Research Ethics, Srinakharinwirot University, Faculty of Dentistry.

The animals were randomly divided into 3 groups as follows: Group I served as untreated controls, Group II comprised 6 rats with plastic appliances, and Group III of 6 rats had fixed orthodontic appliances. Before the experimental procedures, implant markers (Micro Implant, Leibinger, Germany) were placed on the mid-palate nearby the palatine raphe. The animals were anesthesized with an intramuscular injection of ketamine (Keta-hameln®, Hameln Pharmaceuticals GmbH, Hameln, Germany) 50-60 mg./kg. body weight and xylazine (Xylaz®, Farvet, Bladel, Holland) 6 mg./kg. body weight. Local anesthesia (Lidocaine 2%) was injected on the palate before implant and fixed orthodontic appliance insertion.

In group II, rats received clear plastic appliances which were made of materials for the splints (Rocky Mountain Orthodontics, Denver, Colorado, U.S.A.) with 0.5 mm. thickness. A vinyl polysiloxane impression (Sultan Chemists, Inc., Englewood, New Jersy, USA) was taken using a custom tray and the maxillary working model was fabricated by Vel-Mix (Kerr Lab, Orange, California, U.S.A.). The appliance was made from the working model of which the maxillary left first molar was set up mesially 0.5 mm. from the original position. The plastic was heated by a suck down machine (Ministar, Scheu-Dental, Germany) for 5 minutes. The appliance was designed to cover all maxillary teeth and the palate to enhance retention. The incisal area of the appliance was cut through in order to avoid the maxillary incisal undercut. For additional retention, the maxillary teeth on the contra-lateral quadrant and incisors were etched by methacrylated phosphoric acid (Transbond^{tm} Plus Self Etching Primer, 3M Unitek, Monrovia, California, U.S.A.). The appliance was bonded to the teeth by light-curing adhesive (Transbond^{TM} XT Light Cure Adhesive, 3M Unitek, Monrovia, California, U.S.A.), (Figure 1).

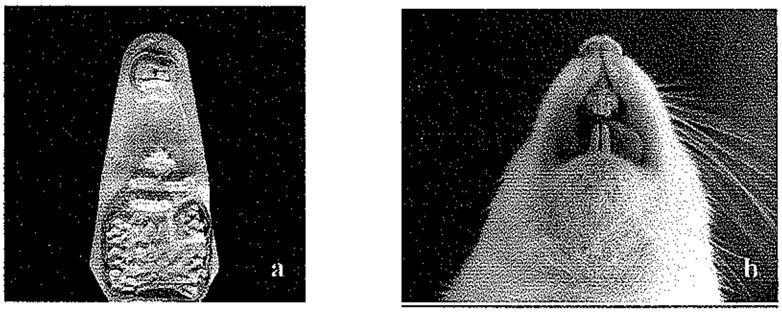


Figure 1 A schematic illustration of a clear plastic appliance moving the maxillary left first molar for 0.5 mm. mesially.(a) The appliance was bonded to the contra-lateral maxillary molar teeth and the incisors.(b)

In group III, maxillary left first molar was moved mesially by a closed-coil spring (Nickel Titanium spring, 0.030" dia., 9 mm., Ormco Corp, Glendora, California, USA.) ligated to the mesial aspect of the molar and through the eyelet on an incisal band as described by Brudvik and Rygh technique[8]. The band was fixed with glass ionomer cement (Protech, Ormco Corp., Glendora, California, U.S.A.), (Figure 2). The force exerted at the time of insertion was 20 g. measured by a Force gauge (ETM Corporation, Monrovia, Canada) calibrated by suspending a known weight. In both experiment groups, mandibular incisal edges were grinded and unsharpened to prevent damages to the appliances.

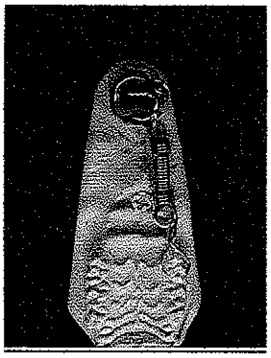


Figure 2 A schematic illustration of a fixed orthodontic appliance moving the maxillary left first molar by a closed-coil spring.

Radiographic procedures

Lateral radiographs and occlusal films (Kodak No. 4 and No. 2, respectively) were taken while the animals being fixed at a head-stat (Figure 3) at 60 KVp, 10 mA, 0.60 seconds and 0.30 seconds, respectively. The distance between mid-sagittal plane of the subject and the film was 5 cm. The magnification factor was 5%. The radiographs were taken on the days of appliance delivery and the days when animal were sacrificed at day 1, day 4, and day 7. The films were traced by a 0.5 sharp pencil on acetate papers. The distance between the implant and mesial aspect of the maxillary left first molar (Imp-Mo), and the implant to labial surface of the maxillary incisors (Imp-Inc) was measured in mm. on the occlusal film. The angle between the palatal plane and the lingual surface of the maxillary incisors (Inc –PP) was measured on the lateral radiographs (Figure 4 and 5)

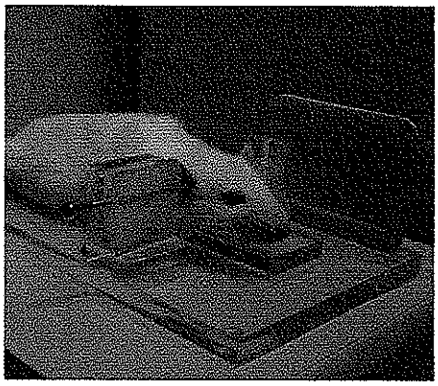


Figure 3 A head-stat for taking a lateral radiographs.

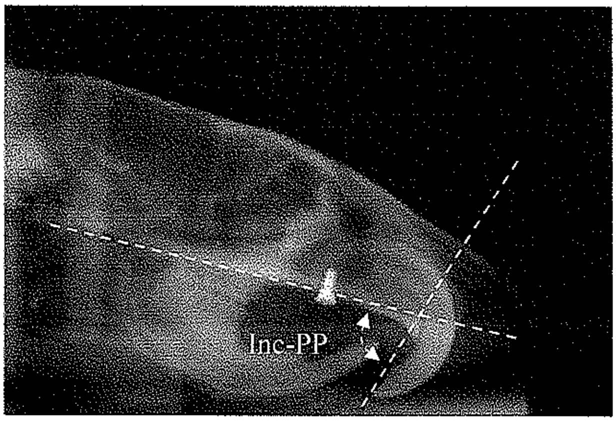


Figure 4 The angle between the palatal plane and the lingual surface of the maxillary incisors (Inc–PP) was measured on the lateral radiographs.

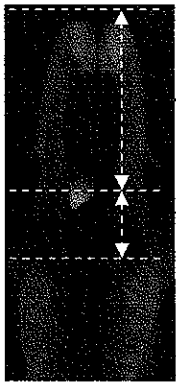


Figure 5 The distance between the implant and mesial aspect of the maxillary left first molar (Imp-Mo), and the implant to labial surface of the maxillary incisors (Imp-Inc) was measured in mm. on the occlusal film

Histological procedures

At the end of each experimental period (day 1, day 4 and day 7), a control and two rats from each experimental group were sacrificed by ether inhalation. After dissection, the maxilla was fixed in 10% buffered formalin solution for 2 days. The specimens were decalcified by 5% Sodium citrate and formic acid and the solution was replaced every day for approximately 2 weeks. The specimens were then divided into the left and right halves including the first, second and third molars and the premaxillary area. After dehydration, they were embedded in paraffin and parasagittal sections of the maxillary left molar teeth were cut at 7 μm. The specimens were lined five sections per slide. The glass slides were stained with haematoxylin and eosin. The area of investigation was the mesial and distal aspects of the mesio-buccal root and distal roots of the maxillary left first molar and adjacent structures, including the inter-radicular alveolar bone septum. The slide showing the greatest length of the root and four adjacent slides covering 60-70 μm. in buccolingual direction were studied by the light microscope under x10 and x40 magnification.

RESULTS

Animals

The animals tolerated the appliances in both types. The weight of the animals in the experiment groups was slightly reduced or stabilized in average during the first 4 days and increased by the first week of the experiment (increased range 1-54 g.)

Histological Observations

Group I as a control group

At the maxillary left first molar roots of the untreated animals, microscopic examination revealed normal periodontal ligament (PDL) structure with narrowing along the apical area (Figure 6). Mesial surfaces of the inter-radicular alveolar bone septum (IRS) and inter-dental septum (IDS), i.e. mainly at the distal alveolar bone of the roots, showed a typical image of bone resorption together with osteoclast (OC) and bone-lining cells. Superficial cementum resorption could be seen and often associated with elongated mononucleated cells.

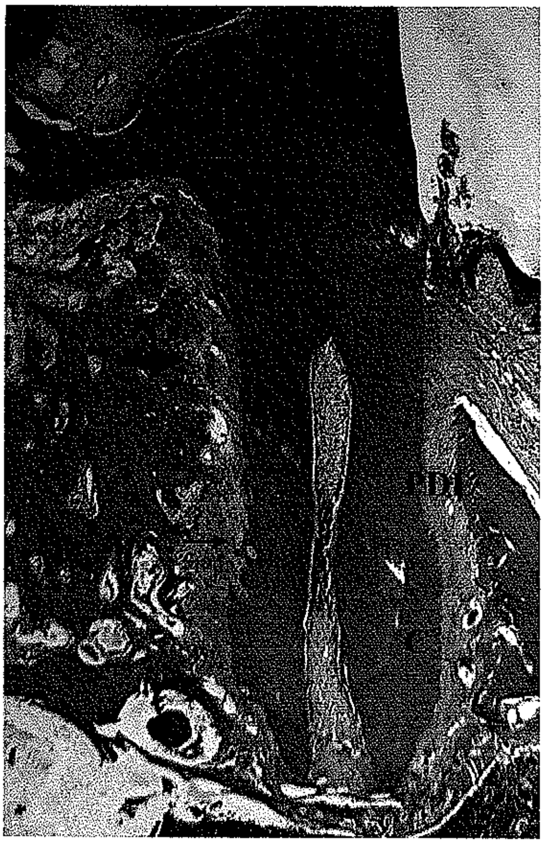


Figure 6 Mesio-buccal root of the maxillary left first molar and neighboring area in a control rat. Bone resorption (dark arrow) associated with osteoclast (OC) and bone-lining cells was noticed primarily at the distal alveolar bone of the root. Superficial cementum resorption (small arrow) could be seen and often associated with elongated mononucleated cells. (T=Tooth, B= Bone, PDL=Periodontal Ligament, C=Cementum)

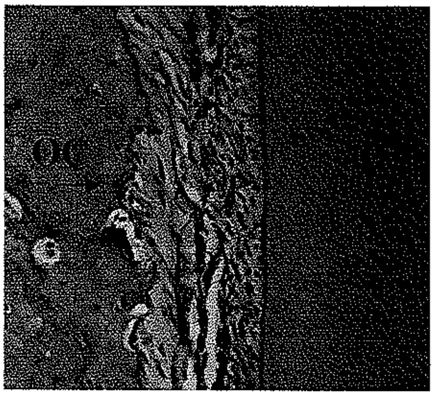


Figure 7 In the control group, mesial surface of the inter-radicular alveolar bone septum showed a typical image of bone resorption together with osteoclast (OC, small arrow) and bone-lining cells

Figure 8 Superficial cementum resorption was noticed and often associated with elongated mononucleated cells (small arrow) in the control group.

Experimental groups

Group II with clear plastic appliances

After one day of experiment, the fibers of the PDL were compressed in the apical and bifurcation area of the roots of the maxillary left first molar (Figures 9-11). By 4 days, the fibers were stretched at the mesial side of the mesio-buccal and distal roots, whereas compression of the fibers was observed at the distal side of both roots (Figures 12-13). After day 7, the PDL of the distal roots and bifurcation presented with further compression at the apical area especially at the distal aspects whereby the PDL of the mesio-buccal root was stretched along the mesial side. (Figure 14)

Figure 9 The fibers of the PDL were compressed in the apical and bifurcation area of the roots of the maxillary left first molar after one day of experiment (small arrows) (Magnification x 4)

Figure 10 Mesio-buccal root of the maxillary left first molar and neighboring area in Group II rat. Compression of the PDL was observed in the apical and bifurcation area as early as day 1 (small arrow). (T=Tooth, B=Bone, C=Cementum, PDL=Periodontal Ligament) (Magnification x 10)

Figure 11 The maxillary left first molar and neighboring area in Group II rat. Compression of the PDL was noticed in the bifurcation area on the first day after force application (small arrow). (Magnification x 10)

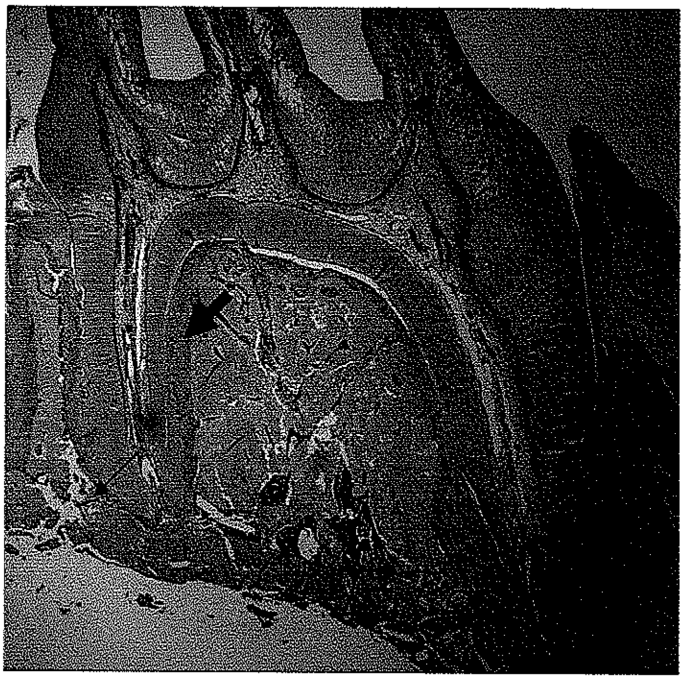


Figure 12 The maxillary left first molar and neighboring area in Group II rat. The PDL was stretched at the mesial side of the roots (dark arrow), whereas compression of the fibers was observed at the distal side of the roots on day 4 (small arrow) (Magnification x 4)

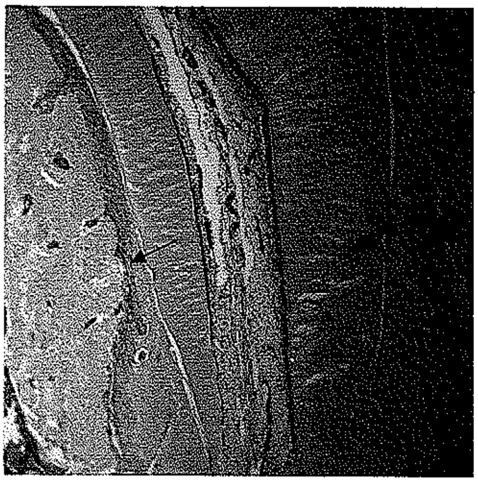


Figure 13 Mesio-buccal root of the maxillary left first molar and neighboring area in Group II rat. The PDL was stretched at the mesial side of the mesio-buccal root (dark arrow), whereas compression of the fibers was observed at the distal side of the root on day 4 (small arrow). (Magnification x 10)

Figure 14 Mesio-buccal root of the maxillary left first molar and neighboring area in Group II rat. The undermining alveolar bone resorption with many OC in large bone-resorptive lacunae appeared highest in day 7 (dark arrow). Multinucleated odontoclasts were observed adhering to resorption lacunae along the root surface (small arrow). (Magnification x 10)

Dentine and cementum resorption as well as resorption lacunae were seen on the root surface as early as 24 hours after force application (Figure 15 (a, b), 16). In hematoxylin and eosin-stained sections, multinucleated odontoclasts were observed adhering to resorption lacunae along the root surface (Figure 14). Numerous mononucleated cells were also observed in root resorption lacunae, often associated with a few multinucleated odontoclasts.

On the alveolar bone surface, the number of OC started to increase after the application of force. By day 1, the OC which was usually followed by active undermining bone resorption was observed on the pressure side in bifurcation, IDS and IRS zones (Figure 16 a, b). The undermining alveolar bone resorption with many OC in large bone-resorptive lacunae appeared highest in day 7 (Figure 14).

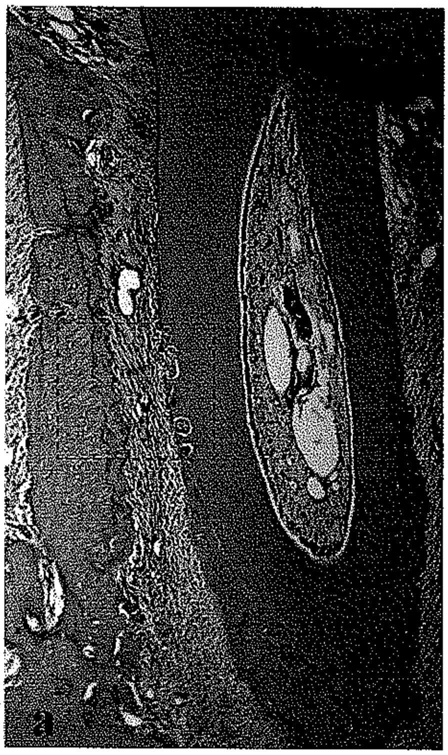
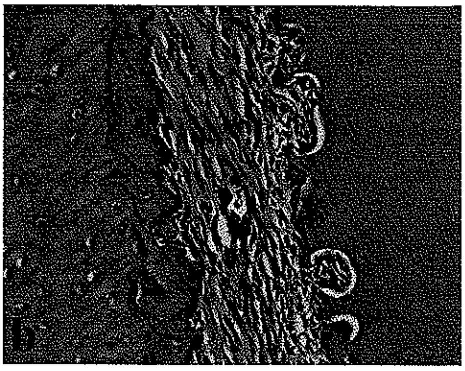


Figure 15, a and b In group II, dentine resorption lacunae were seen on the root surface as early as 24 hours after force application.
(a, Magnification x 4)
(b, Magnification x 40)



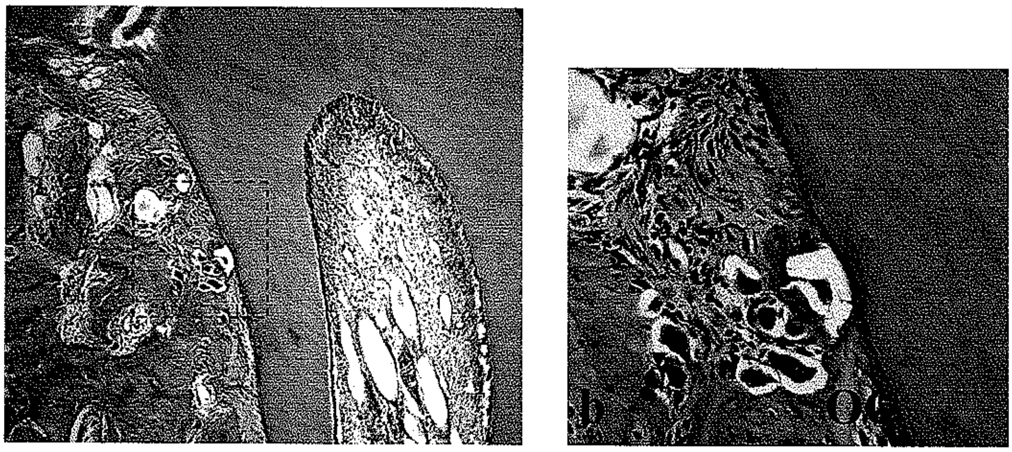


Figure 16 a, b Osteoclast (OC) which was usually followed by active undermining bone resorption was observed on the pressure side in the interdental septum zone (IDS).
(a, Magnification x 4; b, Magnification x 40)

Group III with closed-coil springs

The fibers of the PDL demonstrated signs of disorganization and were compressed at the mesial cervical third and apical of the mesio-buccal and distal roots as well as IRS area after day 1 of the force application (Figure 17). Hyalinized area of the PDL could also be seen in areas of compression (Figure 18). The compression at the cervical mesial aspect and apical area persisted as seen at the mesio-buccal and distal roots whereas the stretched fibers presented at distal aspects of the mesio-buccal and distal roots after day 4 to day 7 (Figure 19 a, b).

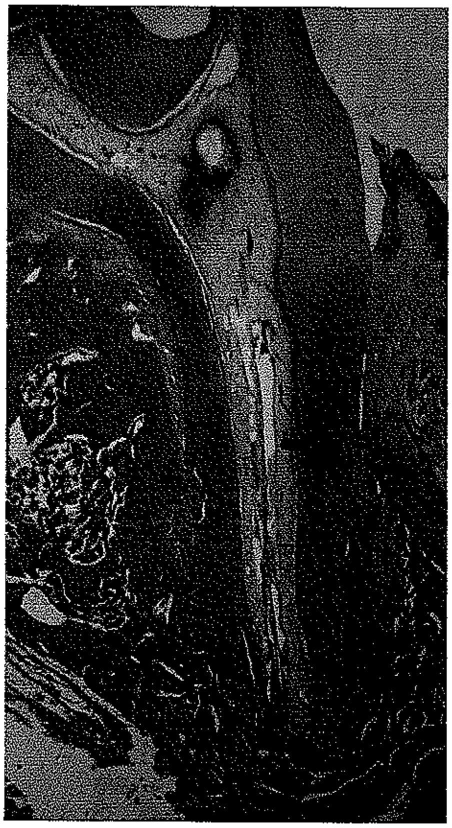


Figure 17 The maxillary left first molar and neighboring area in Group III rat. The PDL was compressed at the mesial cervical third and apical of the mesio-buccal root after one day of the force application (dark arrow) (Magnification x 4)

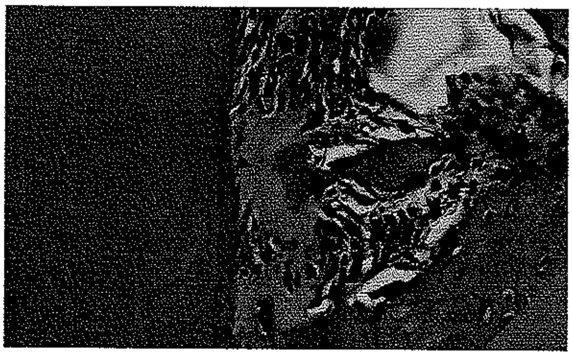


Figure 18 The maxillary left first molar and neighboring area in Group III rat. Hyalinized area (small arrow) of the PDL could also be seen in areas of compression on day one. (Magnification x 40)

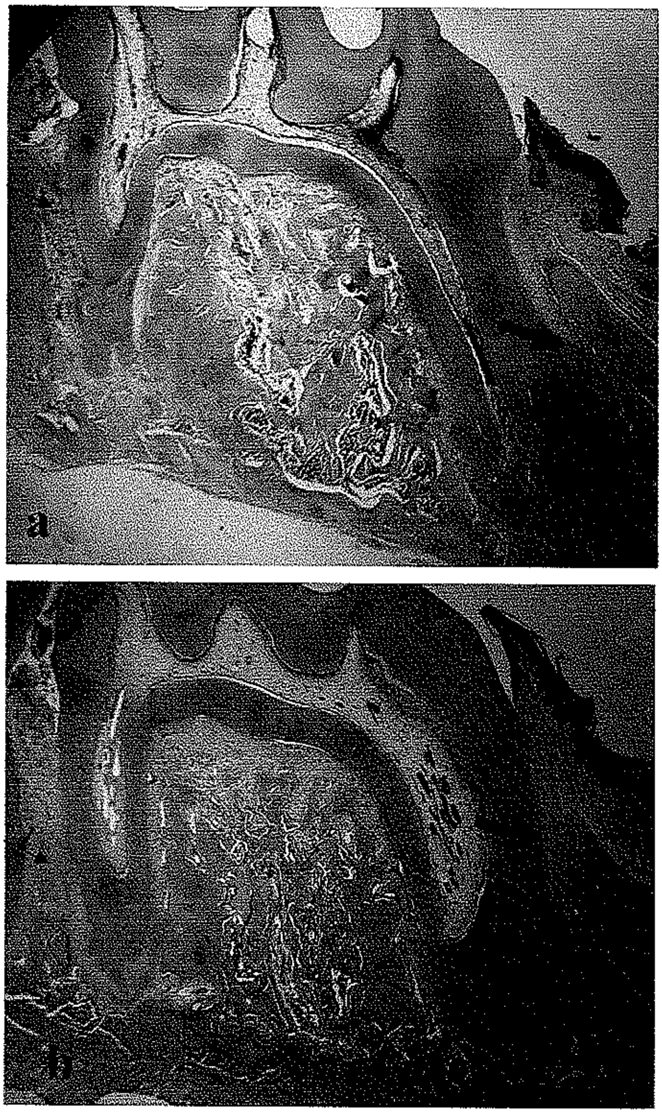


Figure 19 a, b
The maxillary left first molar and neighboring area in Group III rat. The compression at the cervical mesial aspect (dark arrow) and apical area persisted as seen at the mesio-buccal root whereas the stretched fibers presented at distal aspect (small arrow) after day 4 (a) to day 7 (b) (Magnification x 4)

Superficial cementum resorption was also noticed as early as 24 hrs. The multinucleated cells were observed adhering to the resorption lacunae and often associated with numerous mononucleated cells (Figure 20 a, b).

The alveolar bone surface presented with increased numbers of OC and followed by active undermining bone resorption especially along the pressure sides in bifurcation, IDS and IRS zones after day 1. The undermining alveolar bone resorption with many OC in large bone-resorptive lacunae appeared highest in day 7 (Figure 21 a, b). The superficial cementum and alveolar bone resorption were consistent to the findings in group II.

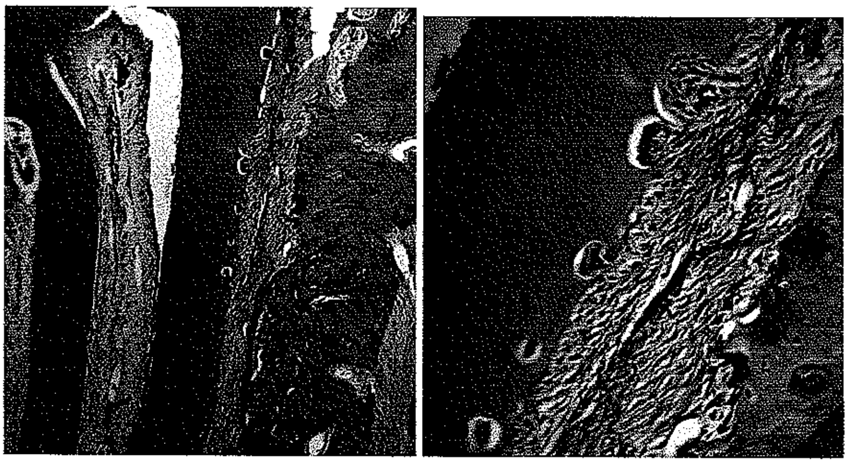


Figure 20 a, b Superficial resorption was also noticed as early as 24 hrs. The multinucleated cells (MN) were observed adhering to the resorption lacunae and often associated with numerous mononucleated cells (MO).
(a, Magnification x 10; b, Magnification x 40)

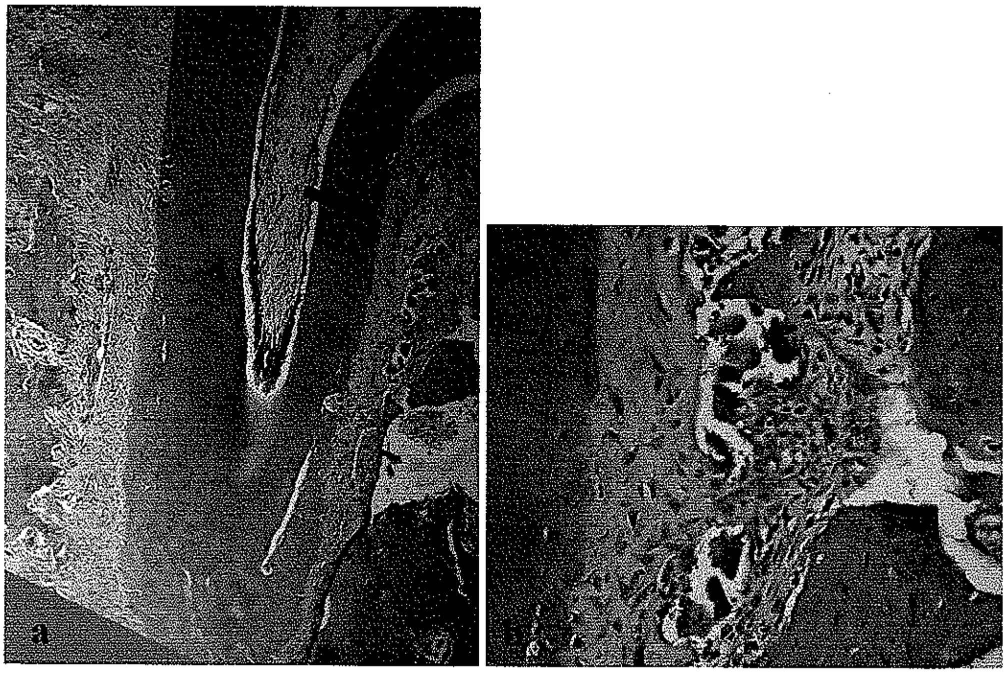


Figure 21 a, b The alveolar bone surface presented increased numbers of OC and followed by active undermining bone resorption especially along the pressure side. The undermining alveolar bone resorption with many OC in large bone-resorptive lacunae appeared highest in day 7.

Radiographic Observations

There were no significant differences of incisal to palatal plane angle (Inc-PP), incisal distance to the implant (Imp-Inc) and molar distance to the implant (Imp-Mo) between the groups at any time interval. (Table 1 and 2) The incisal to palatal plane angle of the rats in the Group 2 and Group 3 tended to tip palatally by day 7.

Table 1 Mean differences of radiographic parameters at each time interval

		Mean Difference (degree, mm.)		
Group	time	Inc- PP	Imp-Inc	Imp-Mo
Group I	Day 1	.0000	.0000	.0000
	Day 4	.0000	.0000	.0000
	Day 7	-2.0000	1.5000	.0000
Group II	Day 1	.0000	.0000	.0000
	Day 4	-1.0000	.2500	.0000
	Day 7	-4.7500	1.2500	.0000
Group III	Day 1	.0000	.0000	.0000
	Day 4	-.2500	.5000	.0000
	Day 7	-8.5000	1.2500	.0000

Table 2 Comparison of mean differences between the groups by Kruskal Wallis Test

	Day 1			4 days			7 days		
	Inc -PP	Imp-Inc	Imp-Mo	Inc -PP	Imp-Inc	Imp-Mo	Inc -PP	Imp-Inc	Imp-Mo
Chi-Square	.000	.000	.000	.750	2.333	1.500	3.600	.667	.000
df	2	2	2	2	2	2	2	2	2
Asymp. Sig.*	1.000	1.000	1.000	.687	.311	.472	.165	.717	1.000

* Kruskal Wallis Test, $p < .05$

DISCUSSION

The histological findings of the control group in this study is consistent to the others' in that small isolated resorption lacunae could be found on superficial cementum and bone resorption was noticed primarily at the distal alveolar bone of the roots.[8, 11, 16, 17] The physiological process and tooth eruption of rat molars have been suggested to explain the small resorption lacunae, variation in root morphology, and distal drift of the maxillary teeth of normal rats. Thus, this was taken into consideration when comparing the results of the experimental groups to the normal controls. In the experimental group III with a closed-coil spring, the findings indicated mesial tipping movement of the maxillary first molar as the force generated by the coil spring was concentrated in the mesial marginal and apical area of the alveolar bone. The compression of the PDL around cervical third at mesial surface and apical area of the maxillary molar roots were found within 24 hrs. and the hyalinized area and undermined resorption of the alveolar bone at the compression site also presented. These findings coincided with other previously published studies in normal growing rats which demonstrated controlled mesial tipping of the maxillary molar after continuous force generated by a coil spring.[8, 10, 11, 13] In this study, the compression of the PDL and alveolar bone resorption found in bifurcation and IRS zones which appeared highest in day 7 also demonstrated some intrusion.

Interestingly, in the experimental group II with a clear plastic orthodontic appliance which the maxillary left first molar was set up 0.5 mm. mesially from the original position, intrusion and tipping movement of the crown were found. These were supported by the findings of compression of the PDL at the apical and bifurcation areas and at marginal level of distal surfaces of the molar roots following with stretched fibers of the PDL at mesial surfaces of the mesio-buccal and distal roots by 4 days. In addition, the hyalinized area of necrotic PDL and undermine alveolar bone resorption occurred mainly in the compressed zones. These findings suggested that initial histo-biomechanics of tooth movement in response to the clear plastic appliance may be intrusion and distal tipping despite the intended mesial movement. The results of the study may be explained by that when the maxillary left first molar of the model was set up for 0.5 mm. mesially, there was a small gap between the maxillary first and second molars. Consequently, this created a flat area of the clear plastic appliance located between the first and second molars when the appliance was fabricated. After insertion in the rats, this flat area possibly caused distal marginal ridge of the maxillary first molar to be initially intruded and later combined with tipping distally. This initial distal tipping tooth movement resulted from the clear plastic appliance was beyond our expectation. Since this preliminary study was conducted for a short duration, it is necessary to further investigate in longer observation whether the maxillary first molar might finally tip mesially after sequential replacement of the clear plastic appliances. With our assumption, tooth movement by orthodontic forces generated by the sequential clear plastic appliances would possibly be so called wiggle movement by initial beginning with intrusion and distal tipping and followed by mesial tipping movement.

Hyalinized area of the PDL on the compression site presented as early as day 1 in both group II and group III which was consistent to the previous studies.[8, 18] Reitan and Kvam[16] pointed out that hyalinized zones were produced as readily and quite early in a small animal such as the rat. The undermined resorption of the hyalinized area associated with the multinucleated cells was noticed few days later.[19] Superficial root resorption lacunae associated with mononucleated cells were seen on the root surface as early as 24 hours after force application in both experimental group II and III. Adherence of the multinucleated odontoclasts to the resorption lacunae along the root surface were also noticed highest by day 7. In group III with a closed-coil spring, though the force application was relatively low for tipping movement, but it still seemed excessive for tooth movement in the rat model. Ren and associates recently reviewed about the rat as a model for orthodontic tooth movement and they proposed to use initial force delivery by a closed-coil spring as low as 10 cN for the maxillary molar unit per quadrant.[7] There were numerous studies of the optimal force for orthodontic tooth movement in animal and human models, however, the optimum force magnitude has not been in general conclusion.[20] In addition, the superficial cementum resorption either during re-activation or after cessation of orthodontic movement in rats has been reported.[17, 21] It is crucial to notice that initial superficial dentine and cementum resorption were observed in group II with the clear plastic appliance as early as orthodontically induced root resorption by a closed –coil spring in the literature.[8, 18, 19] Though, amount of force application generated by the clear plastic appliances has not been able to quantify, results from this study have demonstrated that the clear plastic appliance led to initial intrusion and distal tipping movement, and associated with orthodontically induced root resorption. Superficial root resorption induced by intrusion mechanics specifically resulted from this clear plastic appliance should probably be taken into future consideration as well as quantification of the forces generated by this type of appliance should be brought up to our concern.

Radiographic examination for this experimental tooth movement has not revealed significant difference between the groups. This may be explained by the short treatment time, which was not adequate for radiographic detection of the initial tooth movement observed at a histological level. Longer experiment and observation is implemented in our following study.

The rat model of using a clear plastic appliance partly bonded to the contra-lateral quadrant of the teeth as the present study may not be able to directly extrapolate to the use of sequential removable appliances in human. The clear plastic used in this study may not be categorized as a removable appliance though the appliance on the studied quadrant was left un-bonded and can move to some extent. Thickness of bonding material on the contra-lateral quadrant might also prevent the appliance to be fully seated to the teeth. This might have effects on mechanics and direction of tooth movement. In addition, further investigation with sequential replacement of the clear plastic appliances for a longer experimental period is crutial for future studies.

CONCLUSION

The present investigation demonstrated initial experimental tooth movement in a rat model by a clear plastic appliance. The histological changes of paradental tissues revealed that the clear plastic appliance led to initial intrusion and distal tipping movement of the molar tooth despite the fact that the model set up was intended to move the tooth mesially. Results from the study also indicated initial superficial root resorption which presented similar to the resorption induced by the close-coil spring. This preliminary study described initial tooth movement from forces generated by a single clear plastic appliance. Sequential replacement of the clear plastic appliances in the rat model to further describe on-going histological changes is suggested.

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