Original Article

Preapplication of Orthodontic Forces to the Donor Teeth Affects Periodontal Healing of Transplanted Teeth

Yusuke Suzaki[a]; Yoshiro Matsumoto[b]; Zuisei Kanno[c]; Kunimichi Soma[d]

ABSTRACT

Objective: To investigate how the preapplication of orthodontic forces to the donor teeth affects the periodontal healing after transplantation.

Materials and Methods: The orthodontic force (1.5 cN) was applied to the maxillary right molars of 6-week-old male Spraque-Dawley rats (n = 21) in the experimental side, and the left side of the same animals was used as the control. After 7 days, both right and left maxillary second molars were extracted or replanted. Periodontal conditions were evaluated in the histological specimens 7 days after applying orthodontic force (before and after extraction) and 14 days after replantation.

Results: The application of orthodontic force for 7 days significantly increased the periodontal ligament (PDL) space and also the width of the alveolar socket, which resulted in a rich attached PDL to the root surface of the extracted teeth. Significantly more root resorption was also detected in the control side without preapplication of orthodontic force 14 days after replantation. This root resorption might involve in the disruption of the PDL.

Conclusion: These results suggested that the preapplication of orthodontic force to the donor teeth increased the PDL width and eased the extraction, which might decrease root resorption after replantation.

KEY WORDS: Replantation; Orthodontic force; Periodontal ligament; Root resorption

INTRODUCTION

Autotransplantation has been a major alternative approach in orthodontic practice to replace missing or hopeless teeth. Although the prognosis of autotransplantation has been greatly improved with the prevention of infection in medical science, some unfavorable results such as root resorption or ankylosis[1–6] still remain. It is reported that the successful prognosis of transplanted teeth is dependent on the following factors: the condition of the remaining periodontal ligament (PDL) attached to the extracted donor tooth,[7–9] adaptation of the donor tooth to the sockets,[10] the duration and the method for splinting after transplantation,[11–13] and the timing of endodontic treatment of the transplanted teeth.[9,14] Based on those findings, an attempt to reduce the damage to the PDL may be an acceptable solution for us.

Some clinical[15] or experimental[16] studies have suggested that the preapplication of mechanical stimuli to the donor teeth may slacken the PDL and ease the extraction, which may reduce the damage of PDL. Nevertheless, the jiggling forces used in those experiments were also reported to cause an enhanced rate of destruction of the periodontium, including root resorption.[17,18] Therefore, more appropriate orthodontic forces need to be examined.

In this study, we aim to investigate the effect of preapplying orthodontic forces to the donor teeth and

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also to evaluate the outcomes before and after replantation.

MATERIALS AND METHODS

Animals

Twenty-one 6-week-old male Spraque-Dawley rats (182 ± 18 g) were used in this study. All rats were fed a solid diet and given water ad libitum during the experimental period. Our animal protocols were approved by the local Animal Experimental Committee, and the rats were intraperitoneally anesthetized during experiments, as described previously.[19,20]

Experimental Model

Orthodontic force was applied to the right maxillary molars for the experimental group (n = 21), and the left molars of the same animals were defined as the control group without orthodontic forces (n = 21). Superelastic titanium-nickel (Ti-Ni) alloy wires (Furukawa Electric, Tokyo, Japan), 0.15 mm in diameter, 12 mm in length, were used to exert the orthodontic force. The wires were bent like a double spring to keep the distance of both ends at 5.0 mm. The force was measured by using a load cell extraorally before attaching. The wires exerted initial forces of 1.5 cN when the distance of the both ends was 3.0 mm, and the force was almost constant despite the different amount of activation because of the superelasticity of these Ti-Ni alloy wires. Both ends of an orthodontic wire were inserted in the occlusal sulci of the right first and second molars, and the wires were attached with light-curing adhesive and composite resin, as described previously[19,20] (Figure 1a). To avoid the adhesion between the first and second molars, the composite resin on the second molars was minimized and the resin on the first molars placed mesially. As a result, the wires exerted the reciprocal force between the first and second molars. The composite resin was raised to the height of 0.8 mm on the right and left first molars to avoid occlusal interference of the second molars. Seven days after the orthodontic force was applied, all wires and resin were removed, and the region for replantation was disinfected using povidone iodine.

Following former reports,[11,21,22] both right and left maxillary second molars were shaken buccopalatally step by step using a specially ordered tissue forceps until the mesiobuccal root dislocated 1 mm and was then immediately replanted to the same place exactly (Figure 1b). No postoperative splinting was used. Extraction and replantation were performed with the same procedure using the same forceps by the same person. Based on a previous report,[11] composite resin was mounted 1 mm in thickness on the occlusal surface of both right and left maxillary first molars to relieve the occlusal contacts for 7 days, to protect the injured PDL from traumatic occlusal forces. Seven days after replantation, the resin was removed to recover occlusal stimuli for prevention of the dentoalveolar ankylosis[11] (Figure 1c).

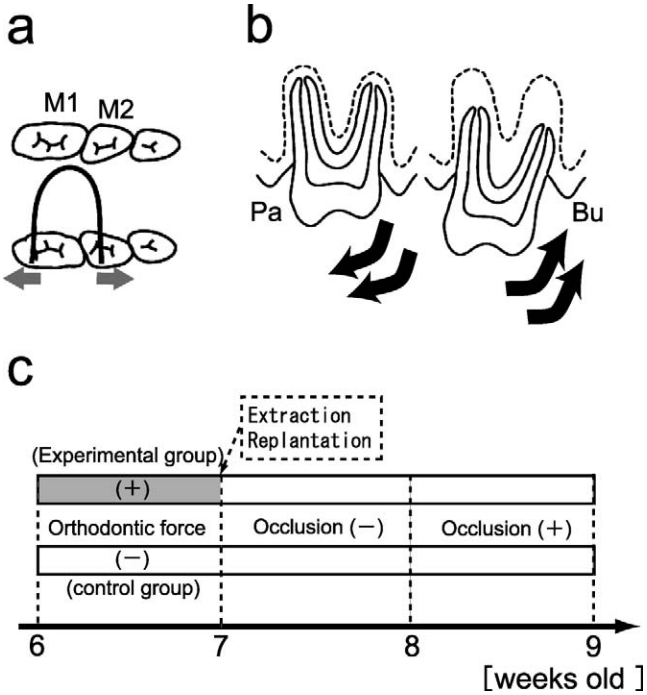


Figure 1. Experimental model in rats. (a) The superelastic Ti-Ni alloy wires produce 1.5-cN orthodontic forces. M1 indicates maxillary first molar; M2, maxillary second molar. An arrow indicates the direction of reciprocal orthodontic force. (b) Frontal views of the replantation procedure in the maxillary M2. The tooth was dislocated palatally (left) and then immediately replanted (right). Pa indicates palatal; Bu, buccal. (c) Experimental schedule diagram. After orthodontic force was applied for 7 days in the experimental group, both M2 molars were extracted and replanted, followed by 7 days of healing without occlusal contact and then 7 days of occlusal contact again.

Histology and Enzyme Histochemistry

Seven days after applying orthodontic force before extraction (n = 4), after extraction (n = 4), and 14 days after replantation (n = 4), the maxillary specimens and the extracted teeth were removed and fixed in 10% neutral buffered formalin. These specimens were decalcified in 10% ethylenediaminetetra–acetic acid solution (pH 7.4) for 4 weeks, and the mesiobuccal root of the second molar was made into 6-μm-thick coronal paraffin serial sections by conventional methods.

The other 18 extracted teeth in nine rats were stained with toluidine blue and observed stereoscopically. The sections for assessment were selected from the center of the root passing through the pulp among

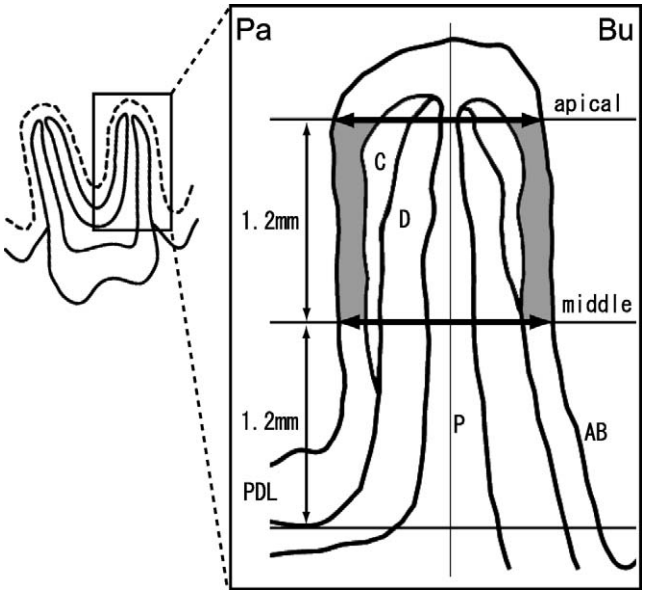


Figure 2. A schematic drawing of the coronal section of the mesiobuccal root of the maxillary second molar. The gray areas were measured as the area of periodontal ligament (PDL). Arrows indicate the width of the alveolar socket in the middle and apical levels. Pa indicates palatal; Bu, buccal; PDL, periodontal ligament; D, dentin; C, cementum; P, pulp; AB, alveolar bone.

the animals. The sections were stained with hematoxylin and eosin, and tartrate-resistant acid phosphatase (TRAP) activity was detected using the azo dye method following counterstaining with 1% methyl green, as reported previously.[23,24] We defined a TRAP-positive multinucleated cell attached on root-resorption lacuna as an odontoclast and root-resorption lacunae including the odontoclast as an active root-resorption lacunae. The apical region of the mesiobuccal roots in the coronal section was observed because the effect of extraction and replantation was typical in the group.

Quantitative Analysis

The area of the PDL, 1.2 to 2.4 mm from the furcation toward the root apex on both buccal and palatal sides, was measured at day 7 of the experiment (before and after extraction; Figure 2). The horizontal length between the inner surfaces of alveolar bone that lay 1.2 mm (middle) and 2.4 mm (apical) away from the furcation at 7 days of the experiment before the extraction was also measured as the width of the alveolar socket. The amount of root exposure without attached PDL cells after extraction was measured as the length of exposed root surface. The number of mesiobuccal roots fractured after extraction was also counted. Furthermore, to quantify root resorption 14 days after replantation, the length of active root-resorption lacunae was measured, as described previously.[24] Image analysis software (Scion Image Beta 4.02, Scion Corporation, Frederick, Md) was used for the histomorphometric analysis.

Statistical Analysis

Comparisons between the orthodontic force and control groups were performed with the Mann-Whitney *U*-test by using the software for statistical analysis (SPSS, SPSS Inc, Chicago, Ill).

RESULTS

The body weight of rats increased consistently during the experimental period. After the application of orthodontic force for 7 days, direct bone resorption could be found on the buccal side of the PDL, and neither hyalinization nor root resorption was detected in the experimental group (Figure 3a). The rats in the experimental group exhibited a significantly wider area of PDL on both the buccal and palatal side (Figure 3b). The width of alveolar sockets at the apical area in the experimental group was also significantly wider (Figure 3c).

When the second molars were extracted after 7 days of applying orthodontic force, the area of PDL was significantly wider in the experimental group than in the control group (Figure 4c). The length of exposed root surface in the experimental group was significantly less on the buccal side, where the PDL space was increased by the orthodontic force (Figure 4d). In addition, during the extraction of the second molars, no fractures of the mesiobuccal roots were found (0/9) in the experimental group, while some root fractures occurred (3/9) in the control group.

At 14 days after replantation, although recovery of the PDL was observed in both the experimental and control groups (Figure 5a,b), the length of active root-resorption lacunae in the apical half of the buccal side in the experimental group was significantly less than that in the control group (Figure 5c).

DISCUSSION

Recently, autotransplantation has been widely performed, and many publications have reported that the survival rate of transplanted teeth may be as high as 90%[13,25–27]; however, some undesirable complications, such as root resorption or dentoalveolar ankylosis, still exist. To improve the prognosis of tooth transplantation, Mine et al[11] focused on the prevention of ankylosis and reported that an occlusal stimulus after transplantation may promote the regeneration of the PDL. Similarly, several reports have suggested that the preapplication of a jiggling force on donor teeth may ease the extraction[28] and reduce root resorption after

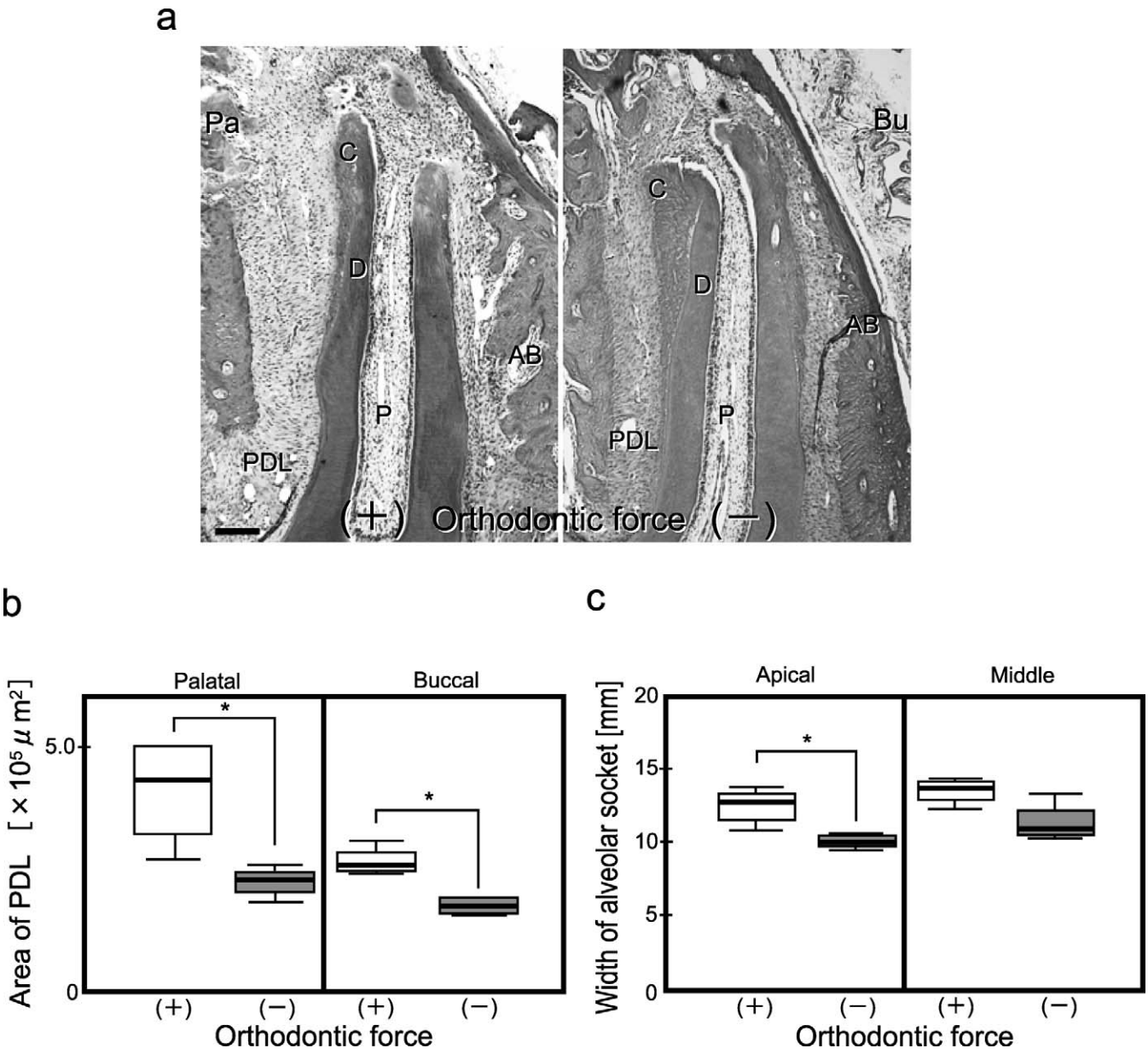


Figure 3. Seven days after the experiment (before extraction). (a) Coronal sections of the mesiobuccal root of the maxillary M2 in the experimental group (left; n = 4) and control group (right; n = 4). Hematoxylin and eosin staining. Pa indicates palatal; Bu, buccal; PDL, periodontal ligament; D, dentin; C, cementum; P, pulp; AB, alveolar bone. Bar = 500 μm. (b) Area of PDL. (c) Width of alveolar socket. The horizontal lines of the box and whisker-plot diagram represent the 95th, 75th, 50th, 25th, and 5th percentiles, ordering from the top. * $P < .05$.

replantation,[16] while Ericsson and Lindhe[17] reported the involvement of jiggling forces in the destruction of the periodontium with periodontitis. With consideration for those understandings, we aimed to validate the use of a light orthodontic force.

The orthodontic force used in this study was provided by Ti-Ni alloy wire and was limited to 1.5 cN, which corresponds to approximately 30 cN for the human first molar.[29] Kohno et al[19] have previously reported that orthodontic forces less than 3.6 cN could move rat molars experimentally without hyalinization and undermining bone resorption. Despite the forces that were applied reciprocally to the maxillary molars in the mesiodistal direction in our study, the enlarged PDL spaces were apparent in both the palatal and buccal sides, and no hyalinization or root resorption could be seen. Figure 3b and c revealed that not only the PDL area but also the width of the alveolar sockets were increased. These changes resulted from the orthodontic force and may have prevented the crushing of the PDL from the destructive pressure between the root and the alveolar bone during extraction and may lead

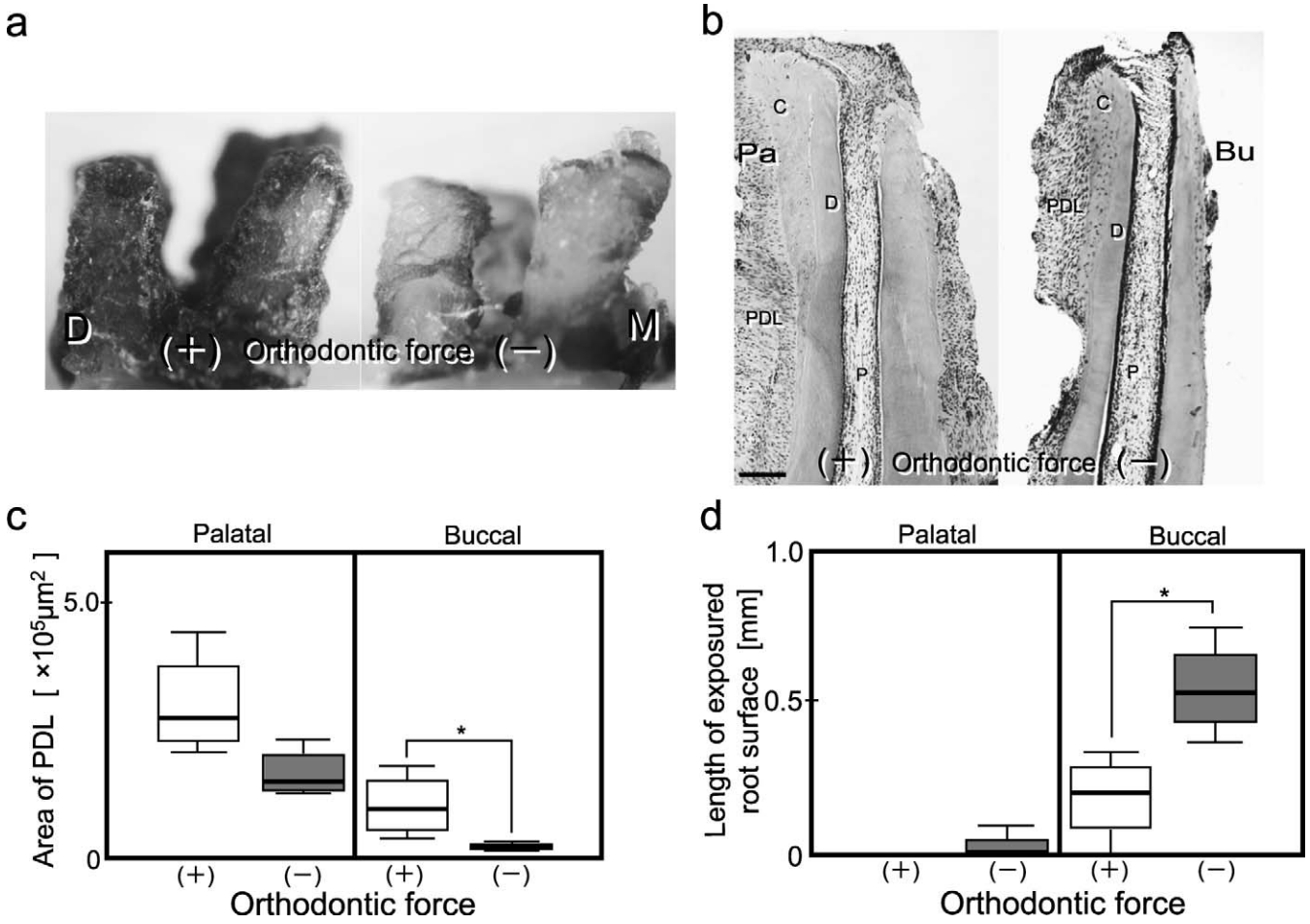


Figure 4. Seven days after experiment (after extraction). (a) Buccal view of the buccal roots of the maxillary M2 stained with toluidine blue in the experimental group (left; n = 9) and control group (right; n = 9). M indicates mesial; D, distal. (b) Coronal sections of the mesiobuccal root of the maxillary M2 in the experimental group (left; n = 4) and control group (right; n = 4). Hematoxylin and eosin staining. Pa indicates palatal; Bu, buccal; PDL, periodontal ligament; D, dentin; C, cementum; P, pulp; AB, alveolar bone. Bar = 500 μm. (c) Area of PDL attached to the extracted tooth. (d) Length of exposed root surface. * $P < .05$.

to a rich volume of PDL attached to the root surface when the tooth was extracted.

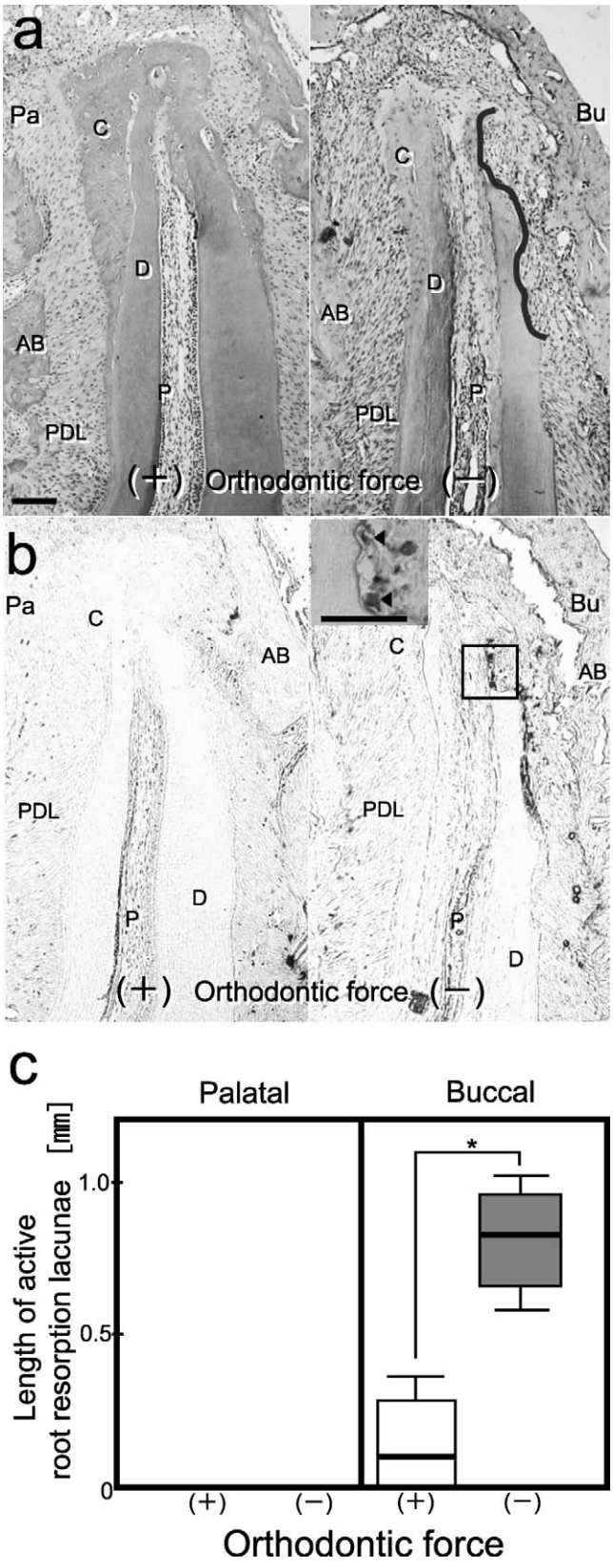
Our results also showed that the increase of root resorption coincided with the disruption of the PDL attached to the root after extraction. The larger the exposed root surface was, the more extensively the root resorption might occur. Many studies have reported that the loss of the PDL from root surface causes root resorption or dentoalveolar ankylosis.[4,9,30,31]

In addition, cementoblasts are involved in the regeneration of resorbed tooth substances. Thus, the extraction of those teeth with a thinner PDL may also have injured the cementoblasts, which resulted in the inhibition of repair of the root after replantation.[32] On the other hand, Herr et al[33] have reported that fibroblasts originating from both the remaining PDL and alveolar bone compartments functioned to repair the periodontium, but cells migrating to the root surface most likely originated from the PDL. This supports the concept that the PDL attached to the extracted tooth could be another key factor for periodontal healing after replantation or transplantation.

Even though Mine et al[11] have noted that occlusal stimuli assist the proliferative activity of PDL cells and promote PDL healing after replantation, easing the extraction of donor teeth may be essential for the better prognosis of replantation or transplantation. When the PDL space was enlarged by the orthodontic force in this experiment, the density of PDL cells was also counted, and no significant difference between the control and enlarged PDL was evident (data not shown). This indicates that the orthodontic force can also promote the proliferation of PDL cells.

CONCLUSION

- These results suggested that the preapplication of orthodontic forces to the donor teeth increased the



PDL space and eased the extraction, which might result in a decrease of root resorption after replantation.

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REFERENCES

1. Andreasen JO. Periodontal healing after replantation and autotransplantation of incisors in monkeys. *Int J Oral Surg.* 1981;10:54–61.
2. Andreasen JO. Analysis of pathogenesis and topography of replacement root resorption (ankylosis) after replantation of mature permanent incisors in monkeys. *Swed Dent J.* 1980; 4:231–240.
3. Andreasen JO. Analysis of topography of surface- and inflammatory root resorption after replantation of mature permanent incisors in monkeys. *Swed Dent J.* 1980;4:135–144.
4. Andreasen JO, Kristerson L. The effect of limited drying or removal of the periodontal ligament: periodontal healing after replantation of mature permanent incisors in monkeys. *Acta Odontol Scand.* 1981;39:1–13.
5. Andreasen JO. Relationship between cell damage in the periodontal ligament after replantation and subsequent development of root resorption: a time-related study in monkeys. *Acta Odontol Scand.* 1981;39:15–25.
6. Andreasen JO. Relationship between surface and inflammatory resorption and changes in the pulp after replantation of permanent incisors in monkeys. *J Endod.* 1981;7:294–301.
7. Blomlof L, Lindskog S, Andersson L, Hedstrom KG, Hammarstrom L. Storage of experimentally avulsed teeth in milk prior to replantation. *J Dent Res.* 1983;62:912–916.
8. Schwartz O, Andreasen FM, Andreasen JO. Effects of temperature, storage time and media on periodontal and pulpal healing after replantation of incisors in monkeys. *Dent Traumatol.* 2002;18:190–195.
9. Cvek M. Treatment of non-vital permanent incisors with calcium hydroxide. I. Follow-up of periapical repair and apical closure of immature roots. *Odontol Revy.* 1972;23:27–44.
10. Oswald RJ, Harrington GW, Van Hassel HJ. Replantation 1: the role of the socket. *J Endod.* 1980;6:479–484.
11. Mine K, Kanno Z, Muramoto T, Soma K. Occlusal forces

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Figure 5. Fourteen days after tooth replantation. (a, b) Coronal sections of the mesiobuccal root of the maxillary M2 in the experimental group (left; n = 4) and control group (right; n = 4). (a) Hematoxylin and eosin staining. (b) Tartrate-resistant acid phosphatase (TRAP) activity. The tracing line indicates the length of active root-resorption lacunae in which TRAP-positive multinucleated cell (odontoclast) attached. Panel shows a magnified area. Pa indicates palatal; Bu, buccal; PDL, periodontal ligament; D, dentin; C, cementum; P, pulp; AB, alveolar bone. Bar = 500 μm. (c) Length of active root-resorption lacunae. * $P < .05$.

promote periodontal healing of transplanted teeth and prevent dentoalveolar ankylosis: an experimental study in rats. *Angle Orthod.* 2005;75:637–644.
12. Andersson L, Lindskog S, Blomlof L, Hedstrom KG, Hammarstrom L. Effect of masticatory stimulation on dentoalveolar ankylosis after experimental tooth replantation. *Endod Dent Traumatol.* 1985;1:13–16.
13. Terheyden H, Gerhardt U, Konig J. Long-term follow-up of tooth transplantation from the functional and periodontal viewpoint [in German]. *Fortschr Kiefer Gesichtschir.* 1995; 40:84–87.
14. Andreasen JO. The effect of pulp extirpation or root canal treatment on periodontal healing after replantation of permanent incisors in monkeys. *J Endod.* 1981;7:245–252.
15. Oshimi H. ''Nemawashi Jiggling'' and ''Gingival Muffler'' in autogenous tooth transplantation. *Nippon Dent Rev.* 1993; 607:65–74.
16. Qu H, Saito S, Morohashi T, et al. Effects of pre-transplantable jiggling force on root resorption of the experimental autotransplanted of teeth in vivo. *Orthod Waves.* 2001;60: 213–225.
17. Ericsson I, Lindhe J. Effect of longstanding jiggling on experimental marginal periodontitis in the beagle dog. *J Clin Periodontol.* 1982;9:497–503.
18. Alwali S, Marklund M, Persson M. Apical root resorption of upper first molars as related to anchorage system. *Swed Dent J.* 2000;24:145–153.
19. Kohno T, Matsumoto Y, Kanno Z, Warita H, Soma K. Experimental tooth movement under light orthodontic forces: rates of tooth movement and changes of the periodontium. *J Orthod.* 2002;29:129–135.
20. Warita H, Iida J, Yamaguchi S, Matsumoto Y, Fujita Y, Domon S. A study on experimental tooth movement with Ti-Ni alloy orthodontic wires: comparison between light continuous force and light dissipating force. *J Jpn Orthod Soc.* 1996;55:515–527.
21. Chen CC, Kanno Z, Soma K. Occlusal forces promote periodontal healing of transplanted teeth with enhanced nitric oxide synthesis. *J Med Dent Sci.* 2005;52:59–64.
22. Kvinnsland I, Heyeraas KJ, Byers MR. Regeneration of calcitonin gene-related peptide immunoreactive nerves in replanted rat molars and their supporting tissues. *Arch Oral Biol.* 1991;36:815–826.
23. Domon S, Shimokawa H, Matsumoto Y, Yamaguchi S, Soma K. In situ hybridization for matrix metalloproteinase-1 and cathepsin K in rat root-resorbing tissue induced by tooth movement. *Arch Oral Biol.* 1999;44:907–915.
24. Sringkarnboriboon S, Matsumoto Y, Soma K. Root resorption related to hypofunctional periodontium in experimental tooth movement. *J Dent Res.* 2003;82:486–490.
25. Andreasen JO, Borum MK, Jacobsen HL, Andreasen FM. Replantation of 400 avulsed permanent incisors. 4. Factors related to periodontal ligament healing. *Endod Dent Traumatol.* 1995;11:76–89.
26. Andreasen JO, Paulsen HU, Yu Z, Schwartz O. A long-term study of 370 autotransplanted premolars. Part III. Periodontal healing subsequent to transplantation. *Eur J Orthod.* 1990;12:25–37.
27. Schwartz O, Frederiksen K, Klausen B. Allotransplantation of human teeth: a retrospective study of 73 transplantations over a period of 28 years. *Int J Oral Maxillofac Surg.* 1987; 16:285–301.
28. Ki HR. The effects of orthodontic forces on the mechanical properties of the periodontal ligament in the rat maxillary molars. *Am J Orthod Dentofacial Orthop.* 1990;98:533–543.
29. Sato T, Iida J, Kurihara S. A histological investigation on the periodontal tissue changes during molar depression in rats. *J Jpn Orthod Soc.* 1984;43:361–372.
30. Andreasen JO. A time-related study of periodontal healing and root resorption activity after replantation of mature permanent incisors in monkeys. *Swed Dent J.* 1980;4:101–110.
31. Lindskog S, Blomlof L, Hammarstrom L. Repair of periodontal tissues in vivo and in vitro. *J Clin Periodontol.* 1983; 10:188–205.
32. Sasaki T, Watanabe C, Shimizu T, Debari K, Segawa K. Possible role of cementoblasts in the resorbant organ of human deciduous teeth during root resorption. *J Periodontal Res.* 1990;25:143–151.
33. Herr Y, Matsuura M, Lin WL, Genco RJ, Cho MI. The origin of fibroblasts and their role in the early stages of horizontal furcation defect healing in the beagle dog. *J Periodontol.* 1995;66:716–730.