

## Tooth Movement in Demineralized Area by Etchant in Rabbits

Bohm Choi<sup>1</sup>, Tae-Gun Kim<sup>2,3</sup>, Seung-Hee Han<sup>2,4</sup>, Yoon-Hee Park<sup>5</sup>, Won Lee<sup>2,5</sup>

1. Department of Oral and Maxillofacial Prosthodontics, Yeouido St. Mary's Hospital, Seoul, Korea
2. Graduate School of Dental Science, The Catholic University of Korea, Seoul, Korea
3. Well Dental Clinic, Seoul, Korea
4. Happiness Dental Clinic, Uijeongbu, Korea
5. Department of Oral and Maxillofacial Surgery, Uijeongbu St. Mary's Hospital, Uijeongbu, Korea

### • Abstract

**Purpose:** Among the facilitation of tooth movement in adult orthodontic treatment methods, surgical approaches are gaining popularity but complications following mechanical bone reduction are a problem. In this study, tooth movement was observed after alveolar bone was chemically demineralized to verify whether tooth movement had been facilitated.

**Materials and Methods:** Twelve rabbits were used. In the experimental group, the alveolar bone of the left first molar area was exposed and demineralized. Thirty seven percents phosphoric acid was applied for 5 minutes for demineralization. The opposite first molar area was used as control. Two teeth were pulled with 200 g force and 4 rabbits each were sacrificed at 3, 7, and 14 days after the force was applied. Histologic examination was done with hematoxylin and eosin and tartrate-resistant acid phosphatase staining.

**Result:** The histologic examination results revealed more bone resorption in the demineralized area. As time passed, the number of osteoclasts increased in the compressed area. The amount of tooth movement was larger in the experimental group compared to the control group but the difference was not statistically significant.

**Conclusion:** The demineralization with etchant resulted in limited bone resorption, more tooth movement and less damage of the cementum after applied orthodontic force.

• Key words : Corticotomy, Dental etchant, Tartrate-resistant acid phosphatase staining

• J Korean Dent Sci. 2012; 5(1) : 37 - 44

### Corresponding Author

**Won Lee, DDS, MSD, PhD**

Department of Oral and Maxillofacial Surgery, Uijeongbu St. Mary's Hospital, 271 Cheonbo-ro, Uijeongbu 480-717, Korea

TEL : +82-31-820-3184 FAX : +82-31-847-2894 E-mail : cmfs21@catholic.ac.kr

Received for publication May 16, 2012; Returned after revision May 30, 2012;

Accepted for publication June 6, 2012

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## Introduction

Many previous efforts have been made to shorten the time required for orthodontic treatment and numerous methods to facilitate tooth movement have been developed and studied. Rapid tooth movement accompanied by surgical treatment has recently gained popularity and is usually being applied in adult orthodontic treatments with delayed tooth movement. Tooth loss due to periodontal disease and decreased proliferation ability of periodontal ligament cells are commonly seen in today's aging population, which cause difficulties in tooth movement in the atrophied bone of the edentulous area in adults and also overall tooth movement is inhibited<sup>1,2)</sup>.

Methods to facilitate tooth movement include medication, mechanical or physical stimulation, laser therapy, and surgical treatment. Among these methods, there is a limitation in local drug delivery with medication<sup>3,4)</sup> and there is difficulty in placing an intraoral stimulant with mechanical stimulation method<sup>5,6)</sup>. While laser therapy is effective at certain wavelengths, some cases showed reverse effects and tooth movement may be suppressed when accompanied with surgical treatment<sup>7-9)</sup>. In view of these limitations, surgical approaches to facilitate tooth movement are gaining interest<sup>9-19)</sup>.

Köle<sup>20,22)</sup> reported in 1959 that corticotomy enables rapid tooth movement by eliminating the continuity of cortical bone that is the main source of resistance for tooth movement and maintains tooth vitality and nutrition supply to bone better compared to osteotomy.

Frost<sup>23)</sup> reported regional acceleratory phenomenon (RAP) for the first time in 1983. This is a local tissue response to various noxious stimulations and is a facilitative phenomenon of biological tissue change that occurs in both soft and hard tissue. Frost<sup>24,25)</sup> especially stressed the role of RAP in biological healing after bone fracture in 1989, reporting that in the healing process following bone fracture, joint surgery, bone graft, and osteotomy, there is 4 times more activity of capillary, osteoblast, and osteoclast formation mediated by precursor cells, capillary, lymph tissue, and hormones. And even when bone reduction or surgery of fracture is successful, bone healing may not occur if an abnormality in biologic function hinders the RAP mechanism. Seboun et al.<sup>26)</sup> reported that selective corticotomy enhances the turnover rate of alveolar cancellous bone and the rate of demineralization and reminera-

lization is enhanced in local bone.

Multiple methods to facilitate tooth movement have been introduced based on these findings. Wilcko et al.<sup>11)</sup> introduced orthodontic treatment accompanied by surgical approaches based on RAP and reported that the amount of tooth movement was three times faster while root exposure was lessened and bone volume was maintained when orthodontic treatment was conducted after selective cortical bone reduction and bone graft. Lee et al.<sup>14)</sup> verified RAP with micro computed tomography in tooth movement accompanied by corticotomy and osteotomy and Wang et al.<sup>15)</sup> reported that more bone morphogenic proteins, angiogenesis and osteoclasts were observed on the mesial side of the tooth after corticotomy compared to osteotomy. Chung et al.<sup>16,17)</sup> showed rapid tooth movement in the anterior and posterior tooth area accompanied by corticotomy in adults and reported that tooth movement was facilitated even with ankylosed teeth or in atrophied cortical bone after tooth extraction.

However, surgical approaches that involve mechanical reduction of cortical bone implies the danger of tooth reduction, bleeding, nerve damage, alveolar bone necrosis, and risk following general anesthesia according to surgery type or area; and a relatively large surgical area may become a burden for both the patient and doctor. So if demineralization of the cortical bone with etchant produces effects similar to corticotomy, the difficulties related with surgical methods can be eliminated and unwanted damage to the surrounding tissue can be lessened, resulting in a reduction of stress for both patient and doctor.

So in this study, dental etchant was applied to the posterior area of maxilla of rabbits and the tooth movement pattern and osteoclastic activity surrounding the moved tooth were verified through histologic analysis.

## Materials and Methods

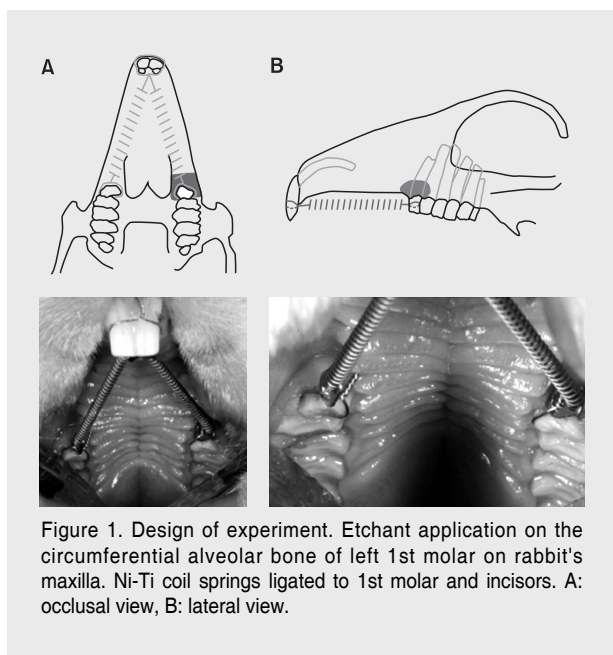
### 1. Experimental Animals and Procedures

Twelve female New Zealand white rabbits with a mean weight of 3.5 kg (range 3.0~4.0 kg) were used as experimental animals and were raised under identical conditions following animal laboratory regulations of the Clinical Research Institute of Uijeongbu St. Mary's Hospital, Catholic University. Each animal was kept in an independent cage that allowed free access to water and food. Permission for this animal study was given by the Institutional Animal

Care and Use Committee of Uijeongbu St. Mary's Hospital, Catholic University (UJA2009-16A).

A mixture of zolazepam 15 mg/kg, tiletamine 15 mg/kg (Zoletil 50, Virbac, Carros, France) and xylazine 5 mg/kg (Xylazine 20 Inj., Kepro BV, Deventer, the Netherlands) was injected into the thigh for general anesthesia. Force was applied in the mesial direction to the maxillary first molar of both sides of the experimental animals. Dental etchant was applied to the exposed alveolar bone surrounding the first molar before force was applied and the first molar on the opposite side was the control.

A flap was lifted around the maxillary first molar and 37% etchant (Bisco etchant, 37% phosphoric acid, pH-0.1, Schaumburg, IL, USA) was applied to a 5 mm area of alveolar bone for more than 5 minutes and then washed with 0.9% normal saline. No treatment was done to the control side but the anterior teeth and maxillary first molar were ligated with 0.009" ligature wire (RMO, Denver, CO, USA) and a force of approximately 200 g was applied with nickel titanium springs (13 mm, Ortholution, Seoul, Korea) (Fig. 1). Gentamicin (Kukje Pharma Ind. Co., Ltd., Seoul, Korea) 4 mg/kg and ketoprofen (Bukwang Pharm. Co., Ltd., Seoul, Korea) 1 mg/kg were injected intramuscularly for 3 days. The animals were sacrificed under deep anesthesia after 3, 7, and 14 days by intravenous KCl (1~2 mmol/kg BW).

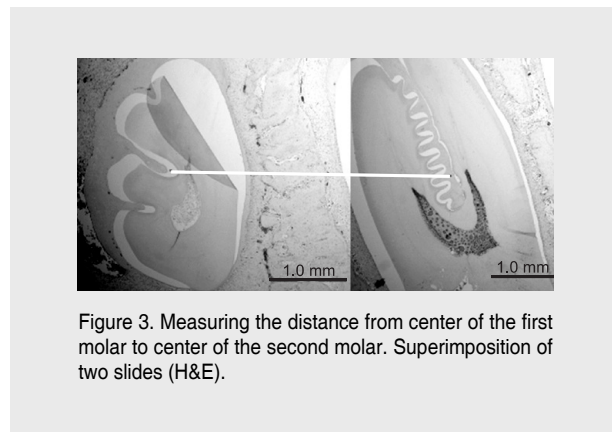
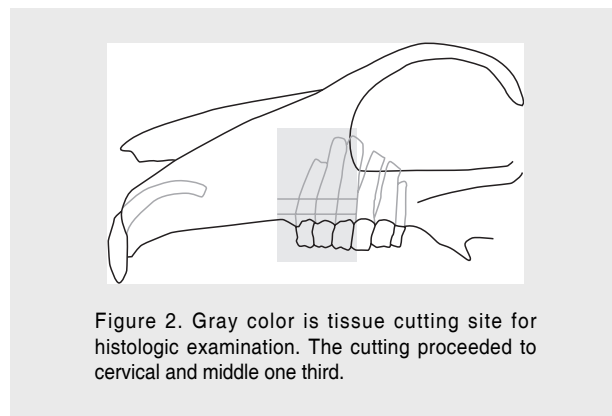


## 2. Histologic Specimen Mounting

The maxilla including the first and second molar and soft tissue harvested from the sacrificed rabbit was immediately fixed with 4% paraformaldehyde. After 48 hours of fixation, demineralization was done for 50 days in 10% ethylenediaminetetraacetic acid (EDTA) on a shaker (Compact Rocker, Fine PCR, Seoul, Korea). The demineralized tissue was embedded in paraffin and the coronal and middle 1/3 was sliced to 4  $\mu$ m thicknesses in a direction parallel to the occlusal surface (Fig. 2). Each specimen was stained with hematoxylin and eosin and observed using a light microscope (IX71 Inverted Microscope, Olympus, Tokyo, Japan).

## 3. Measurement of Tooth Movement

First, cross sectioned images of the tissue slides were acquired with a color digital camera (DP70, Olympus) and then the distance between the center of the root of the maxillary first and second molar was measured using Adobe Photoshop CS2 (Adobe Systems Inc., San Jose, CA, USA)



(Fig. 3). The distance was compared to real measurements at  $\times 40$  magnification and corrected.

#### 4. Tartrate-Resistant Acid Phosphatase (TRAP) Staining and TRAP Positive Cell Count

TRAP staining to identify osteoclasts was done according to the method previously presented by Kim et al.<sup>27)</sup>. The specimens were treated with a mixture of naphthol AS-BI phosphate 4 mg substrate and red violet salt (Sigma, St. Louis, MO, USA) 24 mg for 15 minutes at 37°C. The red violet salt was diluted in 30 ml of 0.1 mol/l acetate buffer (pH 5.2) containing 0.3 mmol/l tartrate (titrated to pH 5.0 using 1N NaOH). Then the specimens were weakly counterstained with hematoxylin.

Histomorphologic analysis was done on the TRAP stained sections to quantitatively evaluate osteoclastic activity.

## Result

### 1. Light Microscopic Findings

The same findings were observed in both experimental and control groups 3 days after application of orthodontic force. The fibrous tissue was narrowed at the mesial area of the first molar and focal spots of bleeding were observed. The distal area of the first molar did not show bleeding and was widened due to tensile strength.

The alveolar bone surface where acid was applied showed

demineralized cortical bone with only fibrous tissue remaining and the surrounding tissue was infiltrated with inflammatory tissue (Fig. 4).

Hyalinization could be found in the compression area 7 days after application of orthodontic force. The mesial and distal side of the alveolar bone of the tooth became more

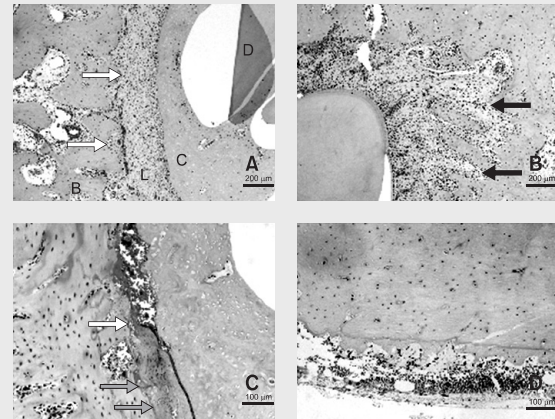


Figure 5. Microphotography after 7 days. (A) Compression side (white arrows). (B) The periodontal ligament is extended and irregularity of the bone is increased at the tension side (black arrows). (C) Hyalinization on the compression area (gray arrows). (D) Partially formed immature bone on the surface of the cortical bone (H&E). D: dentin, C: cementum, L: ligament, B: bone.

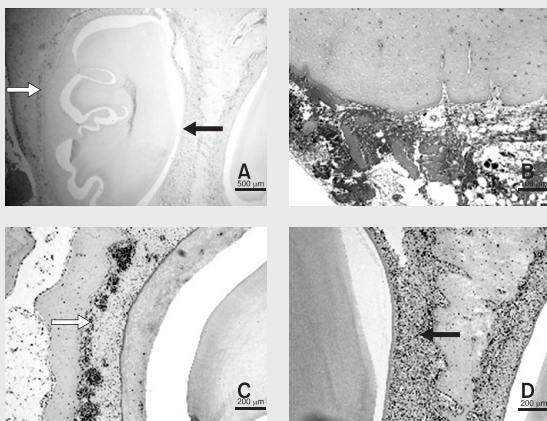


Figure 4. Microphotography after 3 days. (A) Width of periodontal ligaments is narrowed at the compression side (white arrow), and expanded at the tension side (black arrow). (B) Alveolar cortical bone is demineralized. (C) Bleeding tendency on the compression area (white arrow). (D) Expanded ligament at the tension side (black arrow) (H&E).

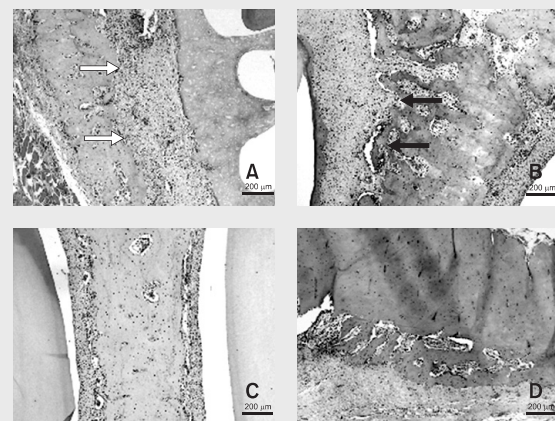


Figure 6. Microphotography after 14 days. (A) The compression side (white arrows). The alveolar bone and small portion of the cementum is absorbed. (B) The tension side (black arrows). New bone formation. (C) Normal interdental bone between 2nd and 3rd molar. (D) Woven bone formation on the surface of the cortical bone (H&E).

irregular and the alveolar bone surface etched with acid showed immature bone formation (Fig. 5).

The alveolar bone revealed some resorption and the narrowed periodontal ligament had become wider while parts of cementum showed resorption at the compression area 14 days after application of orthodontic force. The irregularity of the alveolar bone at the tension side had increased and the interdental space had narrowed due to new bone formation. The demineralized bone surface had replaced to woven bone (Fig. 6).

The control side without acid treatment showed severe resorption of cementum according to the duration of orthodontic force applied, but the acid treated area showed only minimal resorption (Fig. 7).

## 2. Amount of Tooth Movement and t-test

The distance between the center of the root of the first molar and second molar was measured and the mean value was calculated. The results are shown in Table 1. The distance was slightly longer in the experimental group compared to the control group but the difference was not statistically significant.

## 3. Observation of TRAP Positive Cells

Osteoclasts were observed in the compression area and the condition was similar in both the experimental and control group. More osteoclasts were observed as time passed in the

group with orthodontic force applied but the difference was not significant between the two groups during the same period. The demineralized area was healed by woven bone replacement and osteoclasts were observed in the area surrounding the woven bone (Fig. 8).

## Discussion

Etchant used in dentistry was first introduced by Sperber and Buonocore<sup>28)</sup> in 1955. He demineralized enamel using lactic acid, acetic acid, EDTA, and citric acid and then performed radiographic analysis to compare surface condition. He found that demineralization was more complete with EDTA and citric acid.

Studies using etchants were also carried out in bone. Apostolopoulos and Buonocore<sup>29)</sup> compared the level of dis-

Table 1. Distance from center of the first molar to center of the second molar in millimeters

|                      |   | 1    | 2    | 3    | 4    | Means | P-value |
|----------------------|---|------|------|------|------|-------|---------|
| Group 1<br>(3 days)  | C | 3.38 | 3.41 | 3.51 | 3.82 | 3.53  | 0.30    |
|                      | E | 4.39 | 4.30 | 3.31 | 3.47 | 3.87  |         |
| Group 2<br>(7 days)  | C | 3.47 | 3.19 | 3.62 | 3.43 | 3.43  | 0.45    |
|                      | E | 3.51 | 3.16 | 3.84 | 3.78 | 3.32  |         |
| Group 3<br>(14 days) | C | 3.72 | 3.51 | 3.74 | 3.43 | 3.60  | 0.11    |
|                      | E | 3.96 | 3.97 | 3.55 | 3.86 | 3.84  |         |

C: control, E: experiment.

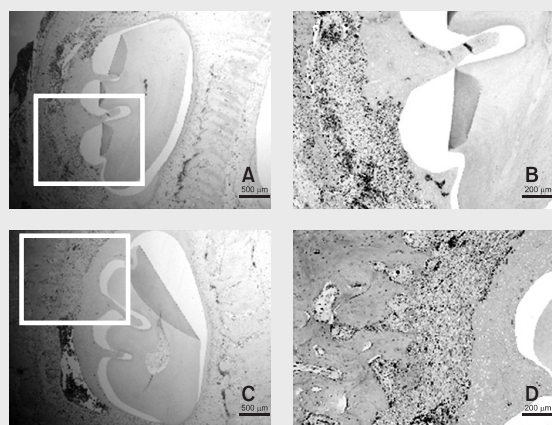


Figure 7. Resorption of the cementum. (A) The control site. (B) The control site shows excessive resorption (enlargement of the white box of A). (C) The experimental site. (D) The experimental site shows a little resorption (enlargement of the white box of C) (H&E).

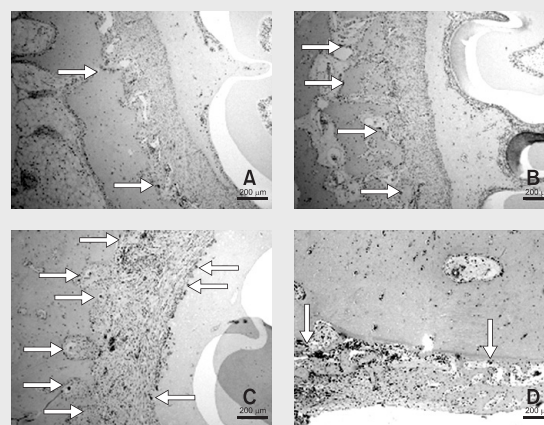


Figure 8. Tartrate-resistant acid phosphatase (TRAP) staining on the compression site (A: 3 days, B: 7 days, C: 14 days) and demineralized surface (D). TRAP-positive osteoclasts have increased with time (white arrows).

solution of hard tissue according to the level of demineralization and reported in 1966 that more dissolution was found with a lower pH and longer application of acid. The level of dissolution was highest in bone and decreased to dentin and enamel, with bone and dentin showing similar values.

Retief<sup>30)</sup> measured the depth of demineralization according to the concentration of phosphoric acid and showed that demineralization occurred to a depth of 10.5~12.5  $\mu\text{m}$  with 10~45% phosphoric acid, but ironically, the depth tended to decrease at concentrations over 50%.

Dental etchant containing 37% phosphoric acid is generally used as a tooth etchant these days and was used in this study with the expectation that it would cause the greatest degree of demineralization. It is possible to apply it locally due to its gel form. The amount of demineralization increases with time but the alveolar bone was demineralized for 5 minutes to avoid complications. This not only helps to shorten surgery time but also lessens the damage to soft tissue and adjacent periodontal tissue from spreading of the etchant.

In humans the rotation center of teeth exist in 1/3 to 1/2 of the area between the alveolar crest and root apex in single rooted teeth and right at the root side of the root furcation in multi-rooted teeth. In the case of rats, the rotation center of the first molar also exists right at the root side of the root furcation and close to the mesial root<sup>31)</sup>. The rotation center is located close to the alveolar crest due to resistance of cortical bone at the cervical region so an area limited to the rooth of first molar and frontal alveolar crest where tooth moved was demineralized in this study.

RAP can be found at the bone surface even when a simple flap is elevated<sup>32)</sup>. Surgical damage approaching the periosteum causes not only bone formation and resorption but also angiogenesis and changes in hemodynamics that contribute to periodontal tissue regeneration and circulation of associated substances<sup>33)</sup>. So based on such findings, more active RAP can be expected to be generated when the bone is demineralized after flap elevation. Iino et al.<sup>10)</sup> carried out corticotomy on beagles and observed decreased hyalinization of the periodontal ligament due to RAP taking place in the alveolar bone and reported that the amount of tooth movement is enhanced for at least 2 weeks.

However, RAP was observed to be limited to the demineralized area in this study and did not facilitate tooth movement. This implies that RAP was generated due to flap elevation and the remaining etchant but the amount was unable to affect tooth movement and the degree of demine-

ralization was not sufficient to generate satisfactory RAP.

In this study, the amount of tooth movement was measured in certain distances regardless of the duration of orthodontic force application. When very strong force is applied, rapid tooth movement is initially observed due to the compression of the periodontal ligament, but the amount of tooth movement is subsequently minimized because of hyalinization of the periodontal tissue<sup>34)</sup>. Hyalinization is a phenomenon that can be observed with a light microscope and is the most common factor that interrupts rapid tooth movement. When the force applied to the tooth presses the alveolar bone wall too strongly, the periodontium does not adapt by tissue remodeling through cell differentiation and proliferation but goes through focal degenerative changes or reacts as aseptic necrosis. The hyalinization area is eliminated by infiltration of cells and vessels from the area surrounding the compression area or by osteoclasts differentiated from adjacent bone marrow and the surrounding intact periodontium. Remodeling of fibrous tissue gradually occurs following the elimination of the remaining hyalinization tissue. The periodontal ligament becomes wider with more abundant cells and enhanced blood circulation than before the initiation of tooth movement, and continued application of force causes direct resorption of the alveolar bone wall<sup>35)</sup>.

More osteoclasts were observed at the compression area compared to the tension area after TRAP staining. The number of osteoclasts increased with the duration of orthodontic force application and could also be observed at the demineralized area. The condition was similar in both experimental and control groups.

In this study, there was no statistically significant difference in the amount of tooth movement after demineralization between the two groups after orthodontic force was applied, but the amount was slightly more in the experimental group and there was less damage of the cementum compared to the control group.

## Conclusion

The demineralization with etchant resulted in limited bone resorption, more tooth movement and less damage of the cementum after applied orthodontic force.

If it would be possible to apply acid for a longer time without tissue complications, tooth movement could be facilitated by the RAP phenomenon based on deep demineralization. This could become an alternative to mechanical

bone reduction and eliminate postoperative complications.

## Acknowledgement

The authors wish to acknowledge the financial support of

the Catholic Medical Center Research Foundation made in the program year of 2009 (5-2009-B0001-00096).

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