Dentoalveolar response during distraction osteogenesis in a rat model

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ABSTRACT

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In distraction osteogenesis, incremental traction of osteotomized bone edges is used to induce growth of new bone and other adjacent tissues such as gingival, skin, and muscle. This study evaluated histological periodontal changes in the surrounding distraction area to establish a rat model with an optimal distraction protocol.

Nineteen left hemi-mandibles of Sprague-Dawley rats were osteotomized. Groups were as follows: Group 1, control group without mandible osteotomy; Group 2, sham group with mandibular osteotomy but without distraction device fixation; Group 3, experimental group with mandibular osteotomy, and mandibular distraction procedure. After 5 days of latency phase, 12 days of distraction phase was followed at a rate of 0.20 mm per day. The consolidation phase was 4 weeks. Distracted distance was measured on CT images obtained immediately after completing the latency period (T0), distraction period (T1), and consolidation period (T2) to confirm long edges were actually separated if the distracted gap was successfully maintained during consolidation. After the T1 and T2 periods all subjects were sacrificed for histological samples.

A total of 2.4 mm was considered as the expected distracted length. However, at the and of T1 and T2, the actual lengths, obtained from the reference points in the micro-CT images, were 2.02 mm and 1.88 mm, respectively. Histologically, new bone formation occurred in shame and experimental groups. Endochondral ossification was observed at the center of distraction gap during the distraction period, while intramembranous ossification was predominant until the fourth week of consolidation. The root resorption with dentin extension observed in the experimental groups was partially repaired during the consolidation period by formation of a thicker layer of cementum.

This model can be applied to various conditions and treatments related to distraction osteogenesis. However, considering that the effect of distraction osteogenesis on root resorption was located on the osteotomy line, surgical procedures should be minimized to reduce negative alveolar bone response.

Key words: distraction osteogenesis, micro CT, alveolar distraction, root resorption rat model

Dentoalveolar response during distraction osteogenesis in a rat model

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I. Introduction

Research on distraction osteogenesis (DO) was initiated in the fields of orthopedics and traumatology in 1905 by Dr. Alessandro Codivilla (Codivilla, 1994). This technique was popularized in 1950 by Cavril Ilizarow, a Russian orthopedic traumatologist who contributed to both the establishment of basic guidelines for DO and the demonstration of its clinical efficacy.

Distraction osteogenesis induces the formation of new bone between bony segments by distracting them away from each other gradually (Nakamoto, Nagasaka et al. 2002). In addition to new bone formation, DO induces the growth of adjacent tissues, such as gingiva, skin, muscle, tendon, and blood vessels.

This procedure has become a treatment option for patients with dentofacial abnormalities, such as maxilla and mandibular hypoplasia, facial asymmetry, and cleft lip and palate (Liou, 2009). During cleft palate closure, the combination of DO and rapid orthodontic tooth movement can help reconstruct the maxillary dentoalveolar defect by approximating the native alveoli and gingiva, thereby minimizing the alveolar cleft fistula and promoting new alveolar bone and gingival tissue (Liou et al., 2000).

Further applications include the formation of new alveolar bone that allows implant placement or tooth movement into the distracted alveolar bone area (Block et al., 1996; Block et al., 1998).

The intra-oral devices used in DO can be either tooth-borne (Block et al., 1995; Niederhagen et al., 1999) or bone-borne (Mommaerts 2001). They have gained popularity because they are easier to use and are more acceptable to patients. Bengi et al. and Dolanmaz et al. Have described a distraction procedure using a rapid palatal expansion screw. This tooth-borne appliance has the disadvantage of increasing the load on the anterior teeth, which are significantly affected (Dolanmaz, Karaman et al. 2003). Therefore, Karakasis and Hadji petrou introduced a new distraction method using a bone-borne appliance with 2 cylindrical distracters (Karakasis and Hadji petrou, 2004).

When the distraction force is applied at an optimal rate, with no surrounding soft tissue complications, new bone tissue forms and undergoes rapid remodeling (Li et al. 2003).

Despite the common use of DO within the craniofacial complex, the procedure protocols, including latency period, distraction rate, and consolidation period, are largely based on those developed for long-bone distraction. Several limitations to the direct application of long-bone DO protocols to craniofacial bones have been demonstrated; craniofacial bones have a more complicated structure and more muscle attachments than do long bones, resulting in the need for more sophisticated 3-dimensional force vectors (Meehan, Morris et al. 2006).

While the goal of DO is to induce bone growth and histogenesis without injury or tearing of structures, it is generally accepted that some root resorption and periodontal changes will occur with any type of orthodontic tooth movement. To avoid tipping and severe root resorption, heavy force and early orthodontic tooth movement are not recommended when teeth are moved through regenerated bone created by DO (Ilizarov 1989).

It has been demonstrated that the tensional stress produced by gradual distraction leads to active histogenesis in the surrounding tissues (Cope et al. 1999). During DO in a dog model, fibroblasts were enriched in periodontal ligaments, bone spicules were actively formed along the direction of the distraction vector, and both apical and lateral surface root resorptions were reported to be minimal (Ai, Xu et al. 2008). However, the cause of root resorption could not be distinguished in this study because orthodontic force was loaded to the teeth along with the DO procedure. The goal of the present study is to histologically evaluate the periodontal changes in the surrounding distraction area in order to establish a rat model with an optimal distraction protocol.

II. Materials and Methods

Animals

Nineteen male Sprague-Dawley rats aged 16 weeks (weight, 450–500 g) were used in this study. All animals were housed in separate cages, with a 12-hour light/dark schedule. All had free access to kibble and water. All experiments were reviewed by the committee on the guidelines for Animal Experimentation of Yonsei University and were performed according to the recommendations and conditions proposed by this committee (Y 09-120).

Device design

The Distraction device consisted of a orthodontic hygienic type jackscrew /Maximum expantion, 7mm/ (Dentaurum, Spingen, Germany) 0.20 mm per quarter turn; 2 Luhr L-shaped surgical microplate /0.8mm x 5 holes/(KLS martin, Tuttlingen, Germany) and 4 micro cortical self-tapping screws /0.8x5mm, n=3 0.8x3mm, n=1/ (KLS martin, Tuttlingen, Germany), was connected by composite resin.

For this study modified L-shaped surgical plates were used. They were pre-bended and then placed in position together with the jackscrew. Especially, to minimize metal exposure of jackscrew during CT scanning, 45 degree distal bend was done at the point where horizontal the and vertical elements meet (Fig. 1).

The rats were anesthetized using intraperitoneal injections of Rumpun (Bayer, Korea) and Zoletil (Virbac Lab, Carros, France) to achieve general anesthesia and 1% lidocaine (0.5mg/100g body weight) for local anesthesia.

After shaving, a single 2 cm incision was made along the inferior border of the left hemimandible skin. The masseter muscle was reflected transversely and the body of the left hemimandible was exposed. The osteotomy design and location of the bone screws were decided based on the information provided by the micro CT data.

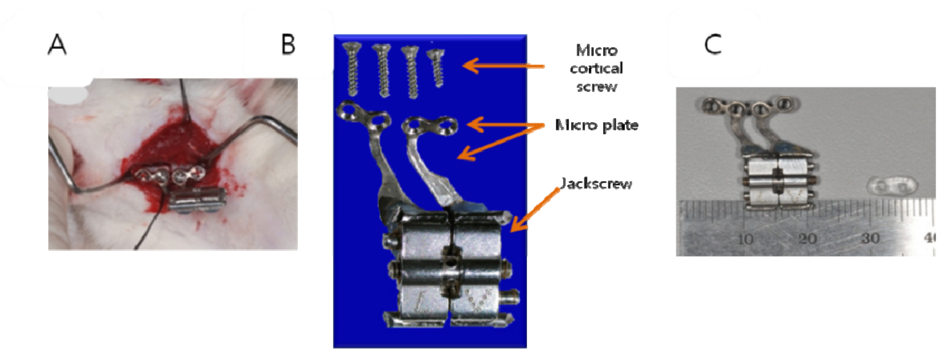

Fig. 1. Distraction device designs.

A. Distractor in placed and osteotomy completed.

B. Components of the distraction device.

C. Final device assembled by jackscrew and pre-bended surgical plate and custom-made Bioplast block to reinforce the mandibular angle area and stable screw maintance.

An periosteal elevator was used to separate the periosteum from the mandible and created an area to position the drill guide over the alveolar bone surface. The guide was used to position four self-tapping titanium bone screws [0.8.x5mm, n =3 (KLS martin, Tuttlingen, Germany); 0.8.x3mm, n= 1] (KLS martin, Tuttlingen, Germany) parallel to the occlusal plane and halfway between the superior and inferior borders of the body of the mandible, so that the osteotomy line could be done between the second and third molars (Fig. 2).

All osteotomy was performed with diamond point bur at 10,000 rpm in an irrigated field. Drill holes were created using a hand drill and copious irrigation. The anterior two screws were placed first once an appropriate position was confirmed. Holes were drilled to respectively, at 10,000 rpm on an irrigated field. Two holes were drilled anterior and one posterior to the osteotomy imaginary line drawn between the second and third molars. The last hole was drilled in the mandibular angle also in an irrigated field. The two anterior self-tapping screws were placed first in order to confirm the appropriate position of the custom made distractor. Then the device was stabilized to the bone by lightly tightening three self tapping, two anterior and one posterior. The osteotomy was performed with a diamond fissure bur at 10,000 rpm in an irrigated field and completed by removing the first posterior screw followed by strong tightening of all four screws.

A complete osteotomy was confirmed by expanding the distractor several turns and observing that bone edges were actually widened. The distractor was then returned to the initial position. After the wound was cleaned with saline irrigation, the muscle and skin layers were returned to their normal position and sutured and around L-plate and device connection site with absorbable silk 3-0 (Vicryl, Ethicon, USA).

The animals were allowed to recover and penicillin G (2000 U/100 g body weight) was administered intramuscularly immediately after the operation. Then all subjects were housed separately and fed with soft diet.

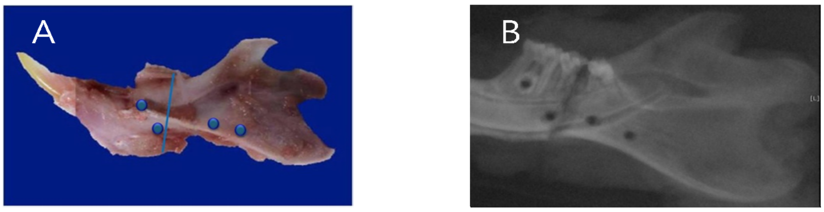

Fig. 2. Osteotomy line

A. Schematic illustraction of osteotomy location (blue line) and position of holes for four miniscrews used for securing the device on the mandible (blue circles).

B. The distraction device applied to the left hemimandible secured by miniscrews. Notice osteotomy line and four drill holes on the X-ray image.

Post-operation care

20 ml Lactated Ringer's solution was injected daily subcutaneously for 14 days following surgery. Neck collar was placed to protect and immobilize the distraction device (Fig. 3). Post surgical management included intraperitoneal injection of antibiotics, and analgesics, trimming of overgrowing incisors to prevent impingement of palatal soft tissue, and daily weighing. All animals had free access to water and soft diet, which was prepared by mixing powdered kibbles and water.

Fig. 3. Rat wearing neck collar to protect and immobilize the distraction device.

Distraction protocol

Subjects were divided into the following groups: Group 1, control group without mandible osteotomy (n = 6); Group 2, sham group with mandibular osteotomy, but without distraction device fixation (n = 6); Group 3, experimental group with mandibular osteotomy, distraction device fixation and application of mandibular distraction protocol (n = 7). After mandibular osteotomy, a 5-day latency phase was followed by a distraction phase of 12 days, during which gradual distractions were performed at a rate of 2.4 mm a day. The distraction phase was followed by a consolidation phase of 4 weeks, during which the external distraction device was maintained in place without any activity. (Fig. 4).

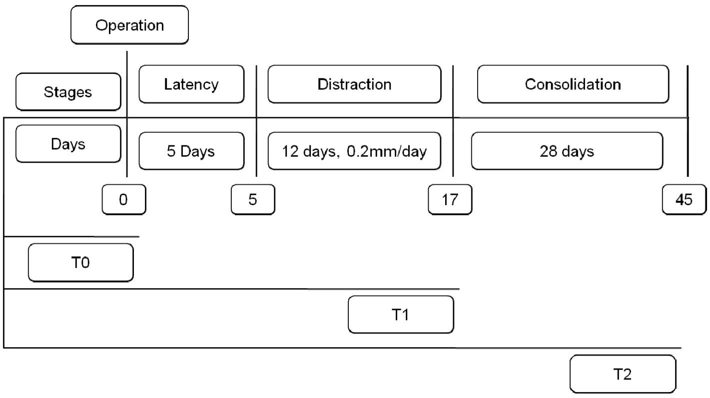

Fig. 4. The sequential stages of rat mandibular distraction osteogenesis experimental protocol.

Tissue preparation

Sacrifice of all subjects, for histologic samples, was done after each experimental stage as follows: T0 (Group 3, n = 1), T1 (Group 1, n = 3; Group 2, n =3; Group 3, n = 3), T1 (Group 1, n = 3; Group 2, n =3; Group 3, n = 3). Then the specimens were fixed on 4% paraformaldehyde for 24 hours, decalcified with 0.5mol/L ethylenediamine tetraacetate (EDTA) 7.5% for 5 weeks. Each left hemimandible was divided axially in two segments, superior (alveolar bone) and inferior (mandibular bone) and then embedded in paraffin. Nine micron sections were mounted on SP 1600 microtome (LEICA, Germany), and haematoxylin and eosin (HE) staining was performed for histological observation.

Micro CT analysis

In vivo micro CT scan of all hemimandibles was obtained using micro CT scanner (SkyScan micro CT 1076, Skyscan, Kontich, Belgium) at a voltage of 100 kV and a current of 100 mA. The micro CT scans were taken at the end of the latency, distraction and consolidation periods. Examinations included scout views, selection of distraction area, reconstruction of image data and analysis. Serial transverse scan images were taken at a resolution of 18 μm. 2-dimensional and 3-dimensional reconstructed by DataViewer Version 1.3.2 (Skyscan, Kontich, Belgium), Nrecon Ver 1.5 (Skyscan, Aartselaar, Belgium) program.

A 3-dimensional region of interest was generated by interpolation between 2 dimensional free-hand selections of the distracted area and excluded the metal part of distraction device to avoid image blurring. Distracted distance was measured on CT images obtained immediately after completing the latency period (T0), distraction period (T1), and consolidation period (T2) to confirm how long the edges were actually separated and if the distracted gap was successfully maintained during consolidation. After the T1 and T2 periods all subjects were sacrificed for histological samples.

III. RESULTS

A. Post-operative Findings analysis

Weight loss was observed in all animals immediately after surgery. It took 15 days to recover their preoperative weight (Fig 5).

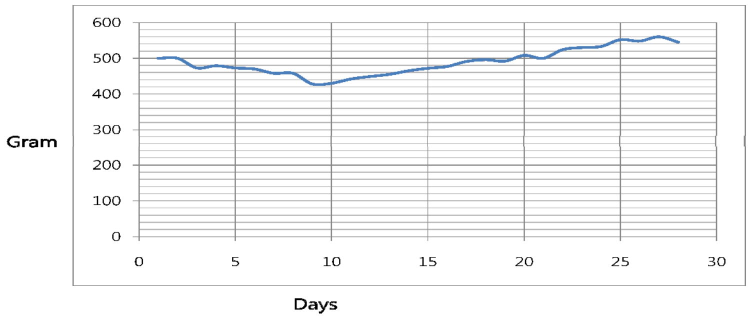

Fig. 5. Diagram showing the change of average weight of all subjects .

B. Micro CT analysis

A the end of T1 and T2 the actual lengths, obtained from the reference points in the micro CT images, were 2.02 mm and 1.88 mm, respectively (Table. 1).

Stages	Distance, mm (mean ± SD)
T0-T1	2.02 ± 0.24
After consolidation (T2)	1.88 ± 0.19

Table. 1. Actual leyths of the expansopn of the jackscrew med in this staty

C. Histology

Root resorption

Control group. Root resorption was not observed in any control subjects (Fig. 6).

Sham group. Small regions of root resorption extending into the cementum-dentin junction area of every third molar were observed in all subjects of the sham group at the end of T2 (Fig. 7). An extension of this root resorption was also observed in poorly distributed areas from the enamel-cement junction to the root apex. Root resorption was also observed in the dentin immediately below the bifurcation area (Fig. 7A). Partial repair, with the resorption cavity partially covered with a layer of hypertrophic cementum, was observed in the fourth week of the consolidation phase (Fig. 7D).

Experimental group. Root resorption extending into the dentin was also observed in most of the experimental subjects (Fig. 8). In a highly magnified view of the compressed side of the distal bone segment, areas of partial resorption of cementum were observed at the mesial radicular surface of the mesial root of every third molar (Fig. 8A, D). Resorption of the cementum layer was also observed at the mesial root apex, while resorption extended into the dentin just below the bifurcation (Fig. 8). Group T2 root resorption reached the cement, but it was less than that in Group distraction period (T1) subjects.

On the tension side, new bone formation accompanied by well organized, extended, dense fibers of periodontal ligament was also observed (Fig 8A). The areas of cementum repair can be seen in (Fig. 8L). This root resorption also extended from the enamel-cement junction to the root apex. The area of cementum repair can be seen in (Fig. 9).

New bone formation.

Experimental group. The central region of the distracted area was filled mainly by fibrous connective tissue with a small amount of cartilaginous formation. By the last day of distraction (T1), the distraction gap was occupied by more fibrous connective tissue (Fig. 10).

At the end of the consolidation period (T2), there was formation of new bone within the distracted gap (Fig. 11). The central part of the distraction gap revealed the presence of cartilaginous tissue (Fig. 11C). The bone remodeling process, characterized by osteoclasts and osteoblasts on the newly formed bone surfaces, was evident. The distraction gap also contained new bone trabeculae, which extended from the osteotomy edge toward the center of the distraction gap and was bridged by new bone.

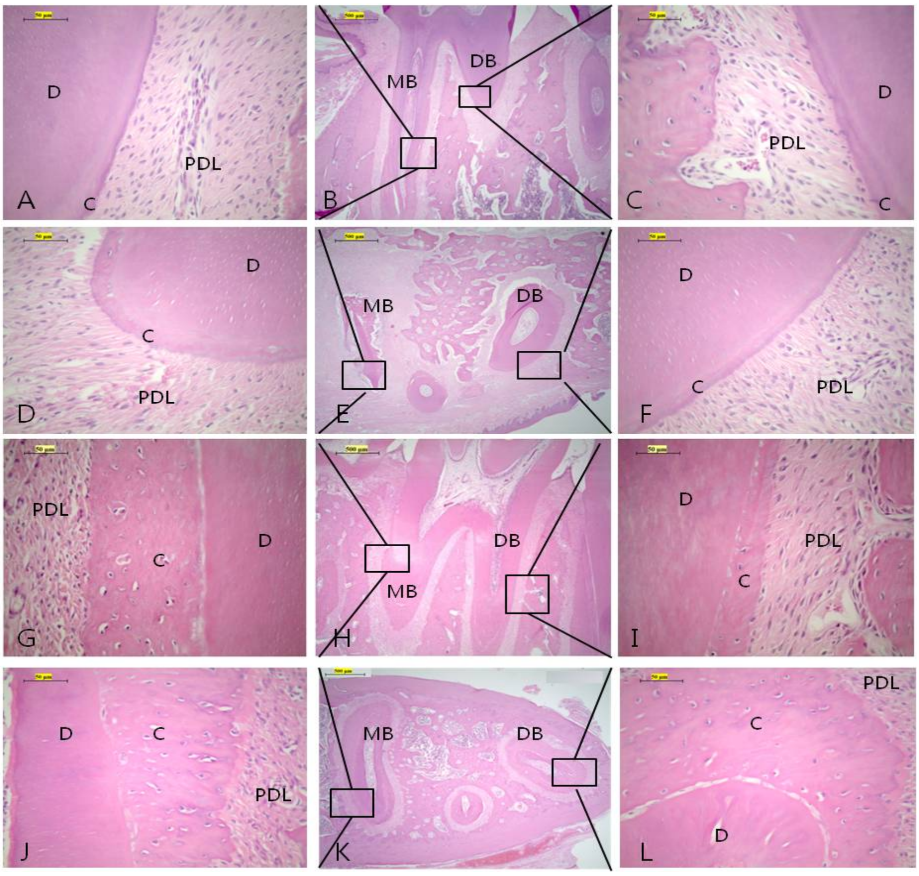

Fig. 6. Histological images of the mandibular third molar of control group subjects at the end of the distraction phase and consolidation phase. Distraction phase (view images A to F). B, E, Third molar sagittal and axial image, respectively (50µm). A, C, Third molar mesial and distal root sagittal image, respectively (500 µm). D, F, Third molar mesial and distal root axial image, respectively (500 µm). Consolidation phase (view images G to L). H, K, Third molar sagittal and axial image, respectively (50 µm). G, J, Third molar mesial and distal root sagittal image, respectively (500 µm). I, l, Third molar mesial and distal root axial image, respectively (500 µm). Along the radicular surface of both mesial and distal roots no areas of resorption were observed. Compared to the distraction phase (A–F), the cementum layer was thickened along the radicular surface of both mesial and distal roots. (DB, disto-bucal radicular surface; MB, mesio-bucal radicular surface; D, dentin; C, cementum; PDL, periodontal ligament; B, bone). H & E staining.

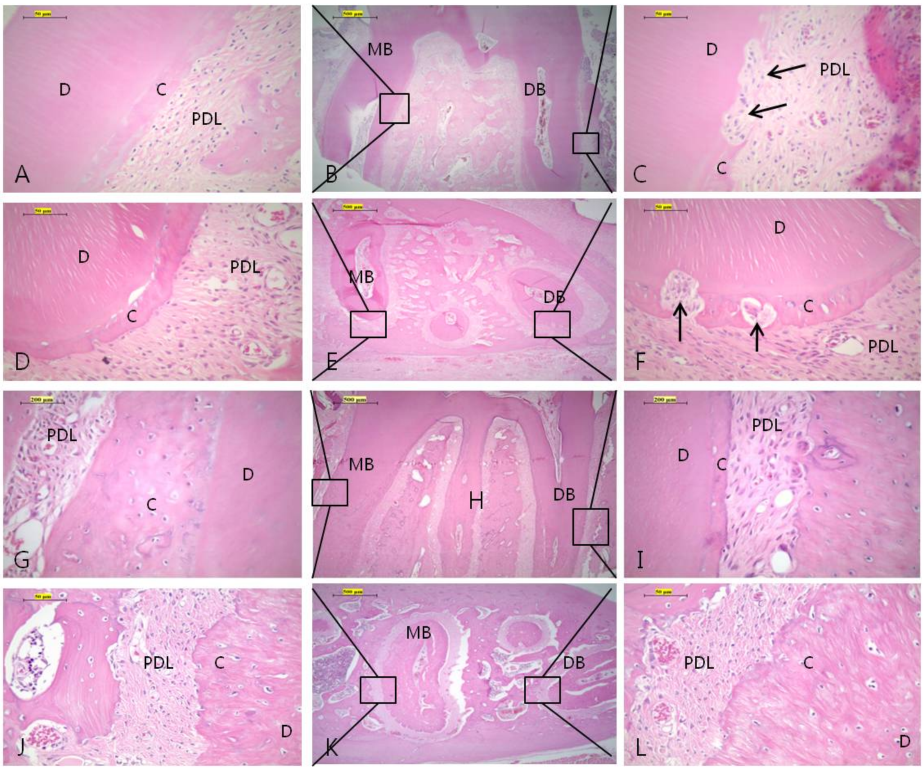

Fig. 7. Histological images of the mandibular third molar of sham group subjects at the end of the distraction phase and consolidation phase. Distraction phase (view images A to F). B, E, Third molar sagittal and axial image, respectively (50 μm). A, C, Third molar mesial and distal root sagittal image, respectively (500 μm). D, F, Third molar mesial and distal root axial image, respectively (500 μm). Along the radicular surface of both mesial and distal roots, poorly distributed areas of root resorption extending from the enamel-dentin junction to the apex were observed. Root resorption into the dentin immediately below the bifurcation area was also observed. Consolidation phase (view images G to L). H, K, Third molar sagittal and axial image, respectively (50 μm). G, J, Third molar mesial and distal root sagittal image, respectively (500 μm). I, l, Third molar mesial and distal root axial image, respectively (500 μm). Along the radicular surface of both mesial and distal roots no areas of resorption were observed. (DB, disto-bucal radicular surface; MB, mesio-bucal radicular surface; D, dentin; C, cementum; PDL, periodontal ligament; B, bone). H & E staining. Partial repair was observed with the resorption cavity partially covered with a layer of hypertrophic cementum.

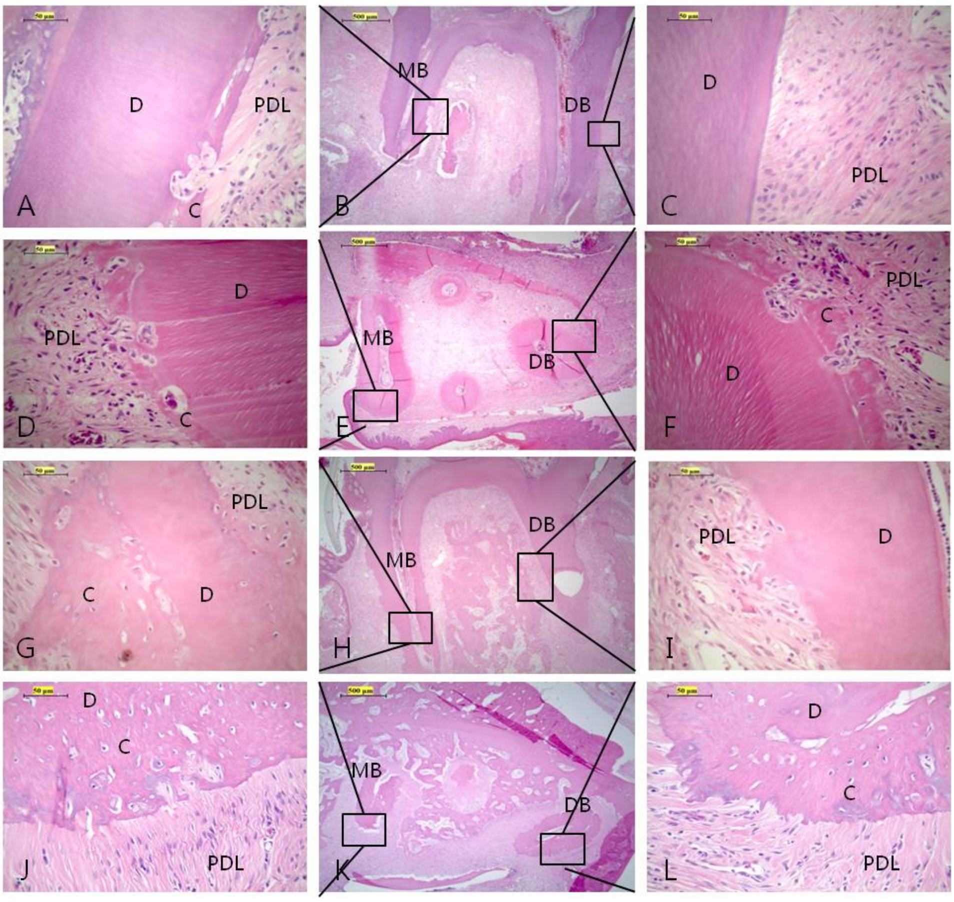

Fig. 8. Histological images of the mandibular third molar of experimental group subjects during the distraction phase and consolidation phase. Distraction phase (view images A to F). B & E, Third molar sagittal and axial image, respectively (50μm). A & C, Third molar mesial and distal root sagittal image, respectively (500 μm). D & F, Third molar mesial and distal root axial image, respectively (500 μm). Root resorption extending into the dentin is observed. In a highly magnified view, cementum resorption is more noticeable (D). Consolidation phase (view images G to L). H & K, Third molar sagittal and axial image, respectively (50 μm). G & J, Third molar mesial and distal root sagittal image, respectively (500 μm). I & l, Third molar mesial and distal root axial image, respectively (500 μm). Along the radicular surface of both mesial and distal roots no areas of resorption were observed. (DB, disto-bucal radicular surface; MB, mesio-bucal radicular surface; D, dentin; C, cementum; PDL, periodontal ligament; B, bone). H & E staining. Compared to the distraction phase, less resorptive lacuna was observed along the root surface (G-L)

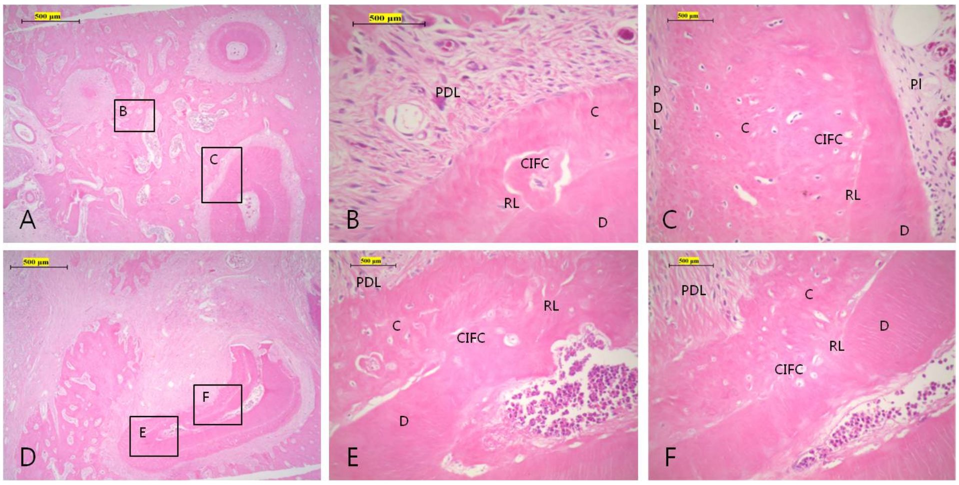

Fig. 9. Histological images of the mandibular third molar of experimental group subjects at the end of the consolidation phase. Mesial and distal root axial image. Areas of cementum repair are seen. (CIFC**,** cellular, intrinsic fiber cementum; D**,** dentin; RL**,** reversal line). H & E staining; magnification bars: 500 µm (A, D), 50 µm (B, C, E, F).

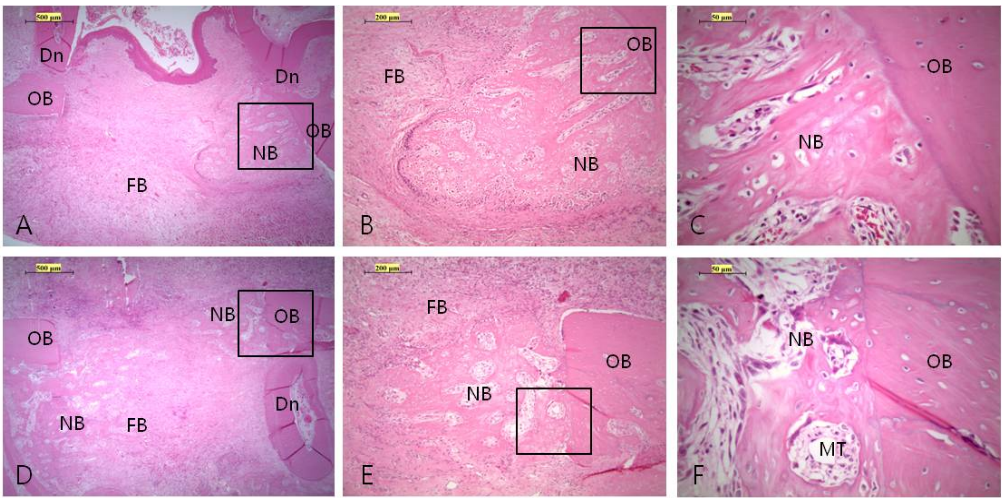

Fig. 10. Histologic images of alveolar host bone and new bone formed between M2 and M3 after 12 days of DO in rat mandible (T1). A–C: mesial and distal root sagittal image; D–F: mesial and distal root axial images. Evidence of bone remodeling is observed among the newly formed bone surfaces. The central area is filled mainly by fibrous connective tissue. NB, new formed bone trabeculae; *OB,* old bone; MT, medullary connective tissue. Magnification bars: 500 µm (A, D); 200 µm (B, E); 50 µm (C, F).

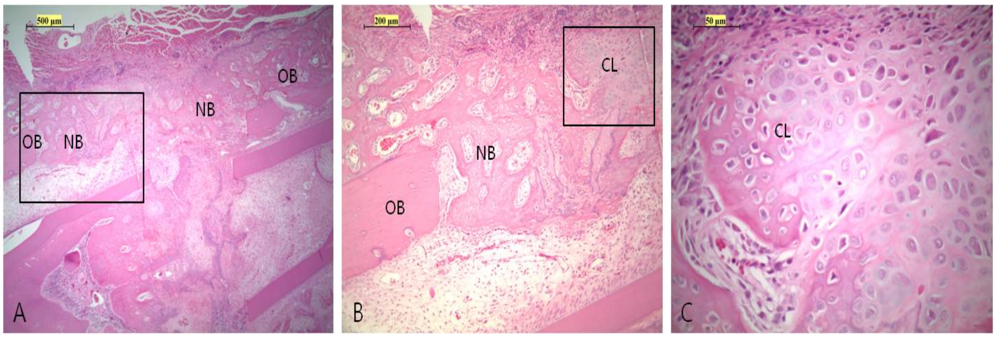

Fig. 11. Histological images of new bone formed between rat mandibular segments after completing 4 weeks of consolidation. The central part of the distraction gap reveals the presence of numerous chondrocytes, which explains the intramembranous bone formation during this phase. *NB,* new formed bone trabeculae; *OB,* host bone; C*L,* chondrocyte. H & E staining. Magnification bars: 500 µm (A); 200 µm (B); and 50 µm (C).

Micro-CT Image Evaluation

At T1, the continuity of the osteotomy line in the sham group was clear (Fig. 12B). In the experimental group, the distracted gap, which showed less radiodensity than the host bone, was clearly observed (Fig. 12D). At T2, the osteotomy line in the sham group could no longer be distinguished. In the experimental group, neither the osteotomy line nor the distracted area could be differentiated from the rest of the bone, not even from the inferior border of the mandible (Fig. 12E).

Root resorption on the compressed side of the distal bone segment was more evident in all subjects during T1 than T2 (Fig. 13E, F).

Figures 14,A and B show the normal anatomy of a control group hemi-mandible, used for comparison to analyze the changes that occurred at T1 and T2 in the sham and experimental groups. The gap between the bone segments in the sham and experimental subjects was obvious during T1. Images of the experimental group show how the gap had been enlarged. (Fig. 14C–D).

After 4 weeks of consolidation (T2), new bone formation could be seen between the bone edges of both sham and experimental subjects (Fig. 14E). In the experimental group, new bone (filling most of the distraction gap) was visible as a homogeneous opaque zone with similar radiodensity as the adjacent mandibular bone (Fig. 14F).

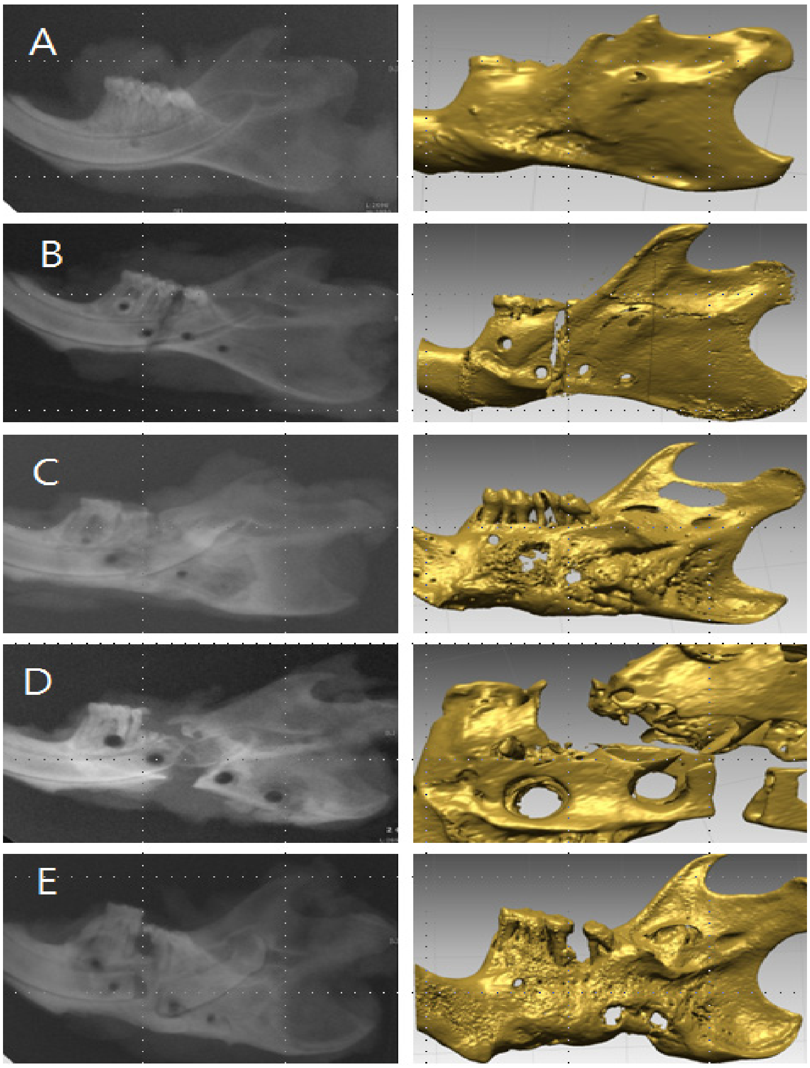

Fig. 12. 3D reconstruction of radicular surfaces. CT images of the first, second, and third mandibular molars, and magnified images showing resorted areas. A. Control group, after 12 days of distraction. B. Control group, after 4 weeks of consolidation. C. Sham group, after 12 days of distraction. D. Sham group, after 4 week of consolidation. E. Experimental group, after 12 days of distraction. F. Experimental group, after 4 weeks of consolidation. MB, mesial buccal root; DB distal buccal root.

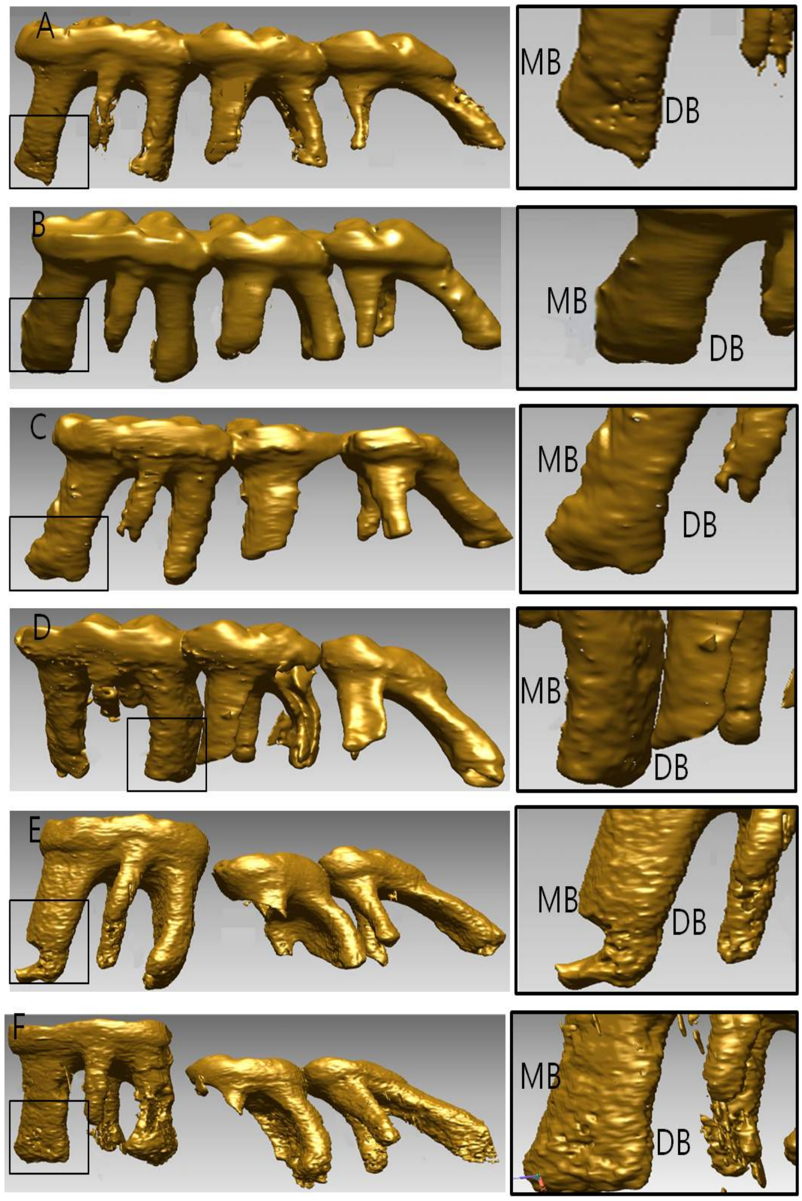

Fig. 13. 3D reconstruction of radicular surfaces CT images of first, second and third mandibular molars and magnified images showing resorted areas. A. Control group, after 12 days of distraction (T1). B. Control group, after 4 weeks of consolidation (T2). C. Sham group, after 12days of distraction (T1). D. Sham group, after 4 week of consolidation (T2). E. Experimental group, after 12 days of distraction (T1). F. Experimental group, after 4 week of consolidation (T2). MB, mesial bucal root; DB distal bucal root.

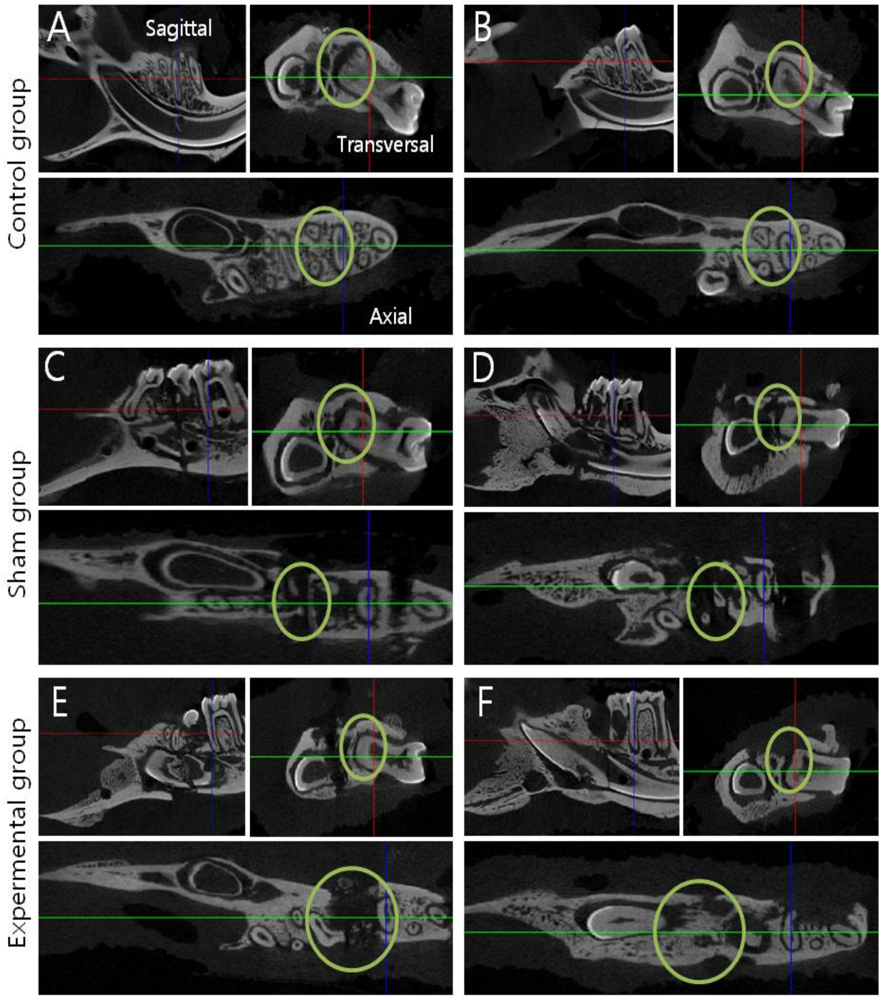

Fig. 14. Axial, sagittal and transversal micro CT images of left hemimandible following sequence of distraction protocol stages. A. Control group, after 12 days of distraction (T1). B. Control group, after 4 weeks of consolidation (T2). C. Sham group, after 12days of distraction (T1). D. Sham group, after 4 week of consolidation (T2). E. Experimental group, after 12 days of distraction (T1). F. Experimental group, after 4 week of consolidation (T2). Inserted circles in A-F transversal images show radicular apex resorption observed in all groups. Inserted circles in E-F axial images show evidence of new bone formation from T1 to T2. MB, mesial bucal root; DB distal bucal root.

IV. DISCUSSION

The purpose of this study was to evaluate the effects of DO on the dentoalveolar structures in rats by histological, micro-CT, and radiographic analysis.

After completing the distraction protocol, a total distracted length of 2.40 mm was expected. However, at the end of T1 and T2, the actual lengths, obtained from the reference points in the micro-CT images, were 2.02 mm and 1.88 mm, respectively. As reported by other authors, relapse of the distraction gap could be explained by a combination of factors, including distraction device failure, poor L-plate fixation, or counteracting masticatory muscle force.

Another interesting finding observed by micro-CT image analysis was the evidence of root resorption and molar tilting during T1 and cementum repair during T2 in most subjects of both sham and experimental groups. At first, it was considered to be the result of the distraction protocol, as reported by Nakamoto et al. (2002). These authors suggested that severe root resorption was caused by the greater bone remodeling process, occurring after rapid movement of teeth into the immature distraction-regenerated bone. However, in our study, a bone-borne rather than a tooth-borne distractor was used; orthodontic movement of both the second and third molars was thus prevented. Occlusal adaptation, local infection, and even the encountered limitations (animal model size, anatomical landmarks, and surgical technique learning curve), were possible contributing factors to the similar pattern of radicular resorption observed between the sham and experimental groups.

Machado et al. concluded that over-compression of the periodontal ligament could lead to hyalinization necrosis and subsequent loss of the ligament. In addition, Wilcko et al. (2001) reported that the rapid accelerating phenomena that occurred after a surgical procedure could affect bone remodeling and healing of the surrounding periodontal tissue. In accordance with the findings reported by both authors, it is possible that over-compression of the periodontal ligament in response to occlusal forces or surgical damage during osteotomy may lead to hyalinization necrosis, ligament loss, and subsequent root resorption.

The healing of bone in the sham subjects, where only osteotomy and fixation of the bone segments was done, took place in the normal manner of fracture healing. However, bone healing in the experimental subjects showed a different pattern, which still is not fully understood. Histological analysis revealed that collagen fibers were aligned along the distraction vector and that trabecular bone structure had been formed by osteoblasts. This structure suggests that intramembranous ossification was induced by tensile force during the distraction phase. At the time of consolidation, the gap between the bone segments was filled with cartilage tissue, demonstrating chondrogenesis. This observation has been reported in other studies (Yasui et al., 1997; Ding Y et al., 2009; Lawler ME et al., 2010) It was observed that while endochondral ossification occurred during the active phase of distraction, mainly at the center of the distraction gap, intramembranous ossification was predominant at the end of the 4fourth week of the consolidation period.

Regarding the cementum repair observed in all groups during T2, similar findings were reported by Owman-Moll et al. (1998). The exposed radicular dentin of most of the resorbed radicular surfaces was repaired with cellular cementum by the end of the consolidation period.

A latency phase of 0–7 days, especially 4–7 days, is indicated for the distraction of facial and alveolar bones, respectively. Even though no latency period is required for large animals, it has been reported that a 5-day latency period is optimal for small animals such as rats and mice (Nakamoto et al., 2002). In this study, the distraction device insertion was followed by a 5-day latency period.

Studies have shown that 1 mm/day is the optimal distraction rate for most experimental animals, except rats. Due to their small size, 0.2–0.6 mm/day is considered an appropriate distraction rate for rats. Paccione et al. and Mehrara et al. (2001) demonstrated that distraction rates of up to 0.50 mm/day produced excellent responses in bone formation. Illizarov suggests that continuous distraction is the optimal condition for the best outcome. Based on these observations, we set our distraction rate to 0.2 mm/day for 12 days with a total distracted length of 2.4 mm.

Previous studies show that improvements have been made in experimental models of DO (Connolly, et al.[2002]; Eski, et al[2005]). Samuchukov et al. (1998) described the importance of device fixation and its maintenance through completion of the consolidation period to the success of DO. At the beginning of our study, there were bone fractures near the bone margins where the miniscrews were inserted, resulting in partial device dislodgement. Therefore, to avoid weakening the bone, at least 2 mm of spare distance was left from its inferior margin. Securing a custom-made resin block together with the L-plate improves the stability of the distraction device and avoids unintentional bone fractures. However, in some subjects, it was inevitable to have slight localized resorption of bone by the pressure resulting from excessive bone contact of the resin block. Similar findings were reported by Connolly et al., (2002)

V. CONCLUSION

During the last several decades, the rat model has been used as a reliable tool for studying DO. In order to develop a quantitative analysis of the effects of dentoalveolar DO, the design of a custom-made device was improved, making its application easier, preventing accidental damage to the susceptible rat mandible, and allowing stable positioning of the device. Successful completion of the DO protocol resulted in the creation of ossifying bone that filled the entire distraction gap.

Apart from good bone regeneration, radicular resorption extending to the dentin was the most common finding observed in all the sham and experimental subjects. This observation can be attributed to the location of the osteotomy line between the molar teeth and its inclusion of a large amount of dental precursor tissue, not to the distraction protocol itself. However, it was not a matter of much concern because cementum repair was evident at the end of the consolidation period, demonstrating the remodeling activity of the distracted regenerating bone. Further studies can be performed to minimize complications by improving surgical techniques and using the advantage of stability provided by the distractor device created for this study.

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국 문 요 약

흰쥐에서 악골신장술에 의한 치아-치조골 반응

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악골신장술은 분리된 뼈 가장자리의 점진적인 견인을 통하여 새로운 골과 인접한 다른 조직을 생성하는 수술로 정의되며 치아치조골 기형 환자의 치료에 악골신장술을 적용하기 위하여, 이번 연구의 목적은 확립된 최적의 신장골 모형에서 치조골 신장 시 치주조직에 대한 변화를 조직학적으로 평가하는데 있다.

19마리의 Sprague-Dawley 쥐 하악골에 골신장을 위해 개발한 맞춤장치를 고정하였다. 실험군과 대조군은 다음과 같이 구성되었다. 그룹 1, 하악골의 절골이 없는 대조군 그룹; 그룹 2, 절골은 시행하였지만 악골신장을 하지 않은 sham 그룹; 그룹 3, 악골신장을 시행한 실험 그룹 이었다. 5일간의 잠복기 이후 24 시간마다의 0.20 mm씩 골신장을 하였고 4주간의 경화기를 거쳤다. 악골신장 거리의 측정

은 하악골의 절단연 사이의 실제 거리를 측정하고. 잠복기 이후(T0)와, 악골신장술 후 (T1), 경화기 후(T2)에 시행되었다. T1과 T2 시기가 지난 후, 조직 절편을 얻기 위하여 실험동물을 희생하였으며 다음과 같은 결과를 얻었다.

1. 2.40 mm 의 골신장 후 실제 골신장거리는 2.02 mm 이었으며, 경화기를 거치면서 1.88 mm 까지 감소하였다.
2. 조직학적 분석결과 하악골의 치조골과 하연의 신장부위에서 막성골화와 연골내 골화의 신생골 결합이 관찰되었다.
3. 상아질까지 침범한 치근흡수가 실험군에서 관찰되었으며 경화기 동안에 백악질 층의 비후와 함께 부분적으로 치유가 되는 양상이 관찰되었다.

본 실험을 통하여 악골신장술은 치조골과 하악골 하연의 동시적인 골신장술 동물 실험 모델을 확립하였다. 골절단으로 인한 신장골 주변 치근의 흡수가 관찰되었으나 골신장에 의한 원인 보다는 외과적인 절단으로 인한 영향으로 판단된다. 따라서 치조골의 부정적인 반응을 줄이기 위하여 외과적 술식은 최소화되어야 한다.

핵심 되는 말: 골 신장술, 치조골 신장술, 재흡수, Micro CT, 백서 모델