MORPHOLOGICAL STUDY ON PERIODONTAL REGENERATION AND ROOT RESORPTION FOLLOWING TOOTH REIMPLANTATION IN RATS

Seung-Jong Lee, Masahiro Matsuura*, Wen-Lang Lin*, Moon-Il Cho*

*Yonsei University, Conservative Dentistry, Seoul, Korea, SUNY at Buffalo, Oral Biology, USA**

ABSTRACT

쥐 치아 재식후 나타나는 치주조직의 치유와 치근흡수에 관한 형태학적 연구

이승종, Masahiro Matsuura*, Wen-Lang Lin*, 조문일*

연세대학교 치과대학, SUNY at Buffalo, Oral Biology, USA*

치근흡수 연구에서 치아재식을 위한 적당한 동물모델을 찾는일은 대단히 중요하다. 본 실험에서는 쥐 대구치의 발치 및 재식을 통해서 치근흡수가 발현되는 모양을 관찰했다. 모두 20마리의 쥐(30일생)에게 0.4% β-APN(aminopropri-onitrile)가 포함된 분말용쥐사료를 5일간 먹인후 케타민 마취후 상악좌우측 제일대구치를 손상을 최소화하여 발치 하였다. 발거된 쥐치아는 0.02M Tris-HCl로 표면처리하여 부착치근막을 제거한후 파라핀왁스로 치근단을 밀폐 하여 재식하였다. 재식후 1, 2, 4, 6, 8 및 12일이 경과된 후 심장관혈을 통하여 쥐를 희생시킨후 3% glutaraldehyde가 포함된 EDTA에서 탈회한후 Epon 포매 하였다. 모든 시편은 유리칼을 사용하여 1미크론 두께로 절편한후 veronal acetate buffer에 녹인 1% toluidine blue로 염색하였다. 파치세포양 세포에 의한 치근흡수는 재식 5 - 7일후 처음 관찰되었다. 재식 12일후에는 치근 전체에 걸친 광범위한 치근흡수가 관찰되었다. 이상의 결과 β-APN을 포함한 쥐사료 식이요법은 쥐 상악제일대구치의 발치와 재식을 용이하게 하여 일정한 치근흡수를 발현시키는데 많은 도움이 된 것으로 판단되었다.

I. INTRODUCTION

The problem of developing a reasonable animal model for root resorption after reimplantation has yet to be solved. Several premises are required for this: the extraction or reimplantation should be technically feasible; the animals should be easy to handle; the result should be reproducible and consistent; the number of animals used for the experiment should be large enough to neutralize the detrimental factors during the experiment; the cost of purchase and maintainance should be reasonable; the morphology of the teeth and surrounding tissues should be similar to those of human, the teeth and the surrounding soft tissues should have a similar healing potential to humans.

The two animal models currently most used for this purpose are the monkey and beagle dog. The major reason for using these two animal models is probably because their teeth are easy to manipulate. The advantage of these models lies in their fairly close reproduction of clinical situations present in humans affected by destructive periodontal disease. The disadvantages include difficulties analyzing the phenomenon due to the complexity of factors which contribute to the conditions. As well, both monkeys and dogs are very costly both to purchase and maintain. This does not allow them to be used on a large scale, which eventually limits the number of experimental specimens. Considering that the basic biological processes in the development of ankylosis and associated root resorptions are the same in all mammals, it

would be of value to develop a method applicable to smaller research animals, such as rats or mice.

Several attempts have been made to utilize the rat and mouse for this purpose. Birkedal-Hanseen (Birkedal-Hanseen 1973) luxated a 10-week-old rat's mandibular first molar and observed three types of resorption: surface resorption, inflammatory resorption, and replacement resorption. The first surface resorption appeared as early as 3 days and grew to have maximum occurrence on the 10th day. Andreasen and Skougaard (Adreasen 1972) described a method in which ankylosis was induced in rat molars by means of surgical damage in the cervical area of the teeth. However, the induced ankylosis was only temporary and was eliminated 10 to 14 days after surgery by resorption. Wesselink and associates (Wesselink 1986) traumatized mouse incisors by local application of liquid nitrogen to the outer surface of the lower jaw and observed extensive root resorptions at the affected area. Later, a cryoprobe technique was introduced (Wheeler 1993, Ng 1990) in order to localize cold trauma to the mouse incisor. Resorption began to appear on the 7th day and gradually increased in size and number until 10th day. No further noticeable increase was observed thereafter. Although localized cold injury provides a simple trauma to develop root resorption and ankylosis, several problems still remain. One is that freezing trauma does not have the same characteristics as routine avulsion trauma. The second is that with this experimental setup it would not be possible to apply the resorption-preventing factors directly to the tooth surface, although the systemic administration is still possible.

Hellsing and Hammarstrom (Hellsing 1993) tested Dakin's solution in order to remove the viable periodontal tissue quickly. They observed the reproducible results with Dakin's solution, although the devitalizing technique did not show any morphological differences. Problems in extraction were dictated. The older the rat was, the more the roots were likely to fracture. No rat older than three months was recommended (Hellsing 1993) for this purpose. In this article, we present an animal model that may be more convenient for screening of a wide range of root conditioning, such as periodontal ligament healing and root resorption after tooth reimplantation.

Ⅱ. METHODOLOGY

1. Animals and extraction of teeth:

A total of 20 Sprague-Dawley female rats with an average body weight of about 100 grams (Harlan Sprague Dawley, Indianapolis, IN) were used. The rats were fed a powdered Purina rat chow diet containing 0.4% beta-aminoproprionitrile(β-APN, Sigma, St. Louis, MO) for five days. β-APN reduces cross-linkage of collagen, thereby reducing the tensile strength of collagen molecules and allowing gentle extraction of teeth with minimal trauma to the surrounding tissues. Under anesthesia with ketamine(0.1cc/animal), the first maxillary molars were extracted using gentle luxation. Bleeding was instantly controlled using cotton tips under gentle pressure. The extracted molars were examined for the integrity of all five roots and the absence of fracture lines.

2. Preparation of teeth:

The extracted molars were washed overnight in sterile water to remove any remaining blood from the root surfaces. The teeth were then incubated for 3 - 4 hours at 37C in 0.02M Tris-HCl, pH 7.4 containing 800 units/ml of bacterial collagenase (Calbiochem, Collagenase, Clostridium histiolyticum, TypeII), 0.2M NaCl and 50mM CaCl2 to denude the root surface from the remnants of periodontal ligament. Approximately 1ml of the above solution was used per 6 molars (a longer digestion time and/or slightly higher concentration of collagenase were sometimes indicated). After digestion, the teeth were washed overnight in sterile water at 4C. The teeth were then left to air dry.

The mesial roots of the maxillary first molars were then demineralized for 1 minute by inserting the mesial roots in a plastic pipet tip which was already filled with saturated citric acid(pH 1.12). The pipet tip was cut to snugly fit the root size. After completion of demineralization, the teeth were washed in sterile water for 30 minutes, dehydrated for three

minutes in 95% and 100% ethyl alcohol and air dried. In order to prevent any communication between the canal space and the surrounding tissue, the apex of the mesio-buccal root was completely sealed in paraffin wax. A heated, sharp instrument was applied to melt the inner side wax of the canal wall to ensure better sealing. The teeth were then stored at 4℃ until the next use.

3. Reimplantation:

Right after the extraction, a previously prepared tooth(obtained from the preceding animal) was reimplanted in the freshly extracted socket under the dissecting microscope. The teeth were held in place and the bleeding was controlled by applying the cotton tips over the reimplanted tooth with gentle pressure. Pressure was maintained until all bleeding completely stopped. Following reimplantaion, the rats were fed a powdered chow diet without -APN for the whole experimental period. This was to minimize tooth loss and to reduce excessive chewing trauma to the surrounding periodontal tissues.

4. Tissue processing:

Rats were sacrificed at 1, 2, 4, 6, 8 and 12 days after reimplantaion by heart infusion with 2.5% glutaraldehyde in 0.1M sodium cacodylate buffer of pH 7.4. The maxillae were removed and fixed in Karnovsky's fixative for an additional 3 - 4 hours, then demineralized at room temperature in EDTA containing 3% glutaraldehyde. At completion of demineralization, the specimens were washed with 0.1M sodium cacodylate buffer, postfixed with 1% OsO4 in s-collidine buffer for 90 minutes, dehydrated by a graded series of ethanol, and infiltrated and embedded with Epon mixture. Polymerization was accomplished at 60C for 48 hours. For light microscopic observation, 1 micron sections were cut on a Reichert Ultracut with a glass knife and stained with 1% toluidine blue in veronal acetate buffer.

Ⅲ. RESULTS

From light microscopic observation 1 day after reimplantation, the wound space was predominantly filled with coagulum(Fig. 1). The remaining gingival connective tissue was relatively intact and was attached closely to the coagulum(Fig. 1a). The apical region of the wound area was also filled with coagulum and was additionally infiltrated with inflammatory cells(Fig. 1b). The remaining PDL had dilated blood vessels, and fibroblasts from the PDL had already started migration into the wound space. At 2 days after reimplantation, the coagulum filling the wound space primarily became rich in fibrin as shown in Fig. 2. In this stage, the gingival connective tissue kept intact(Fig. 2a) and the fibroblasts were migrating actively toward the coagulum(Fig. 2b), the alveolar bone(Fig. 2c), and the root surface(Fig. 2d). In the area of Fig. 2e, PDL fibroblasts also migrated into the wound area. The apical portion of PDL was already healed by this time and was composed of numerous PDL fibroblasts and collagen fibers(Fig. 3f).

At 3 - 4 days after reimplantation, the lower half of the PDL was relatively well healed(Fig. 3c). Even though numerous fibroblasts and newly formed collagen fibers were observed in this area, they demonstrated a parallel arrangement to the root surface. The upper portion of the PDL showed many fibroblasts filling the wound area between the alveolar bone and the gingival connective tissue(Fig. 3b). The cervical wound area still contained coagulum and showed the least progress in wound healing(Fig. 3a). The infiltrating fibroblasts and fibrin were still seen. At 5 - 7 days after reimplantation, the overall healing of the periodontal ligament was improved and characterized by the presence of numerous fibroblasts and collagen fibers between the cells (Fig. 4). However, the fibroblasts in the PDL still showed a parallel arrangement to the root surface, and the apical portion of the root started to demonstrate root resorption by cementoclast-like cells(Fig. 4b). The healing of the gingival connective tissue in the cervical tissue in the cervical area was also improved(Fig. 4a). At 12 days after reimplantation the wound space was completely filled with numerous fibroblasts and collagen fibers(Fig. 5), and the junctional epithelium tightly sealed the connective tissue at the dentino-enamel junction(Fig. 5a). Extensive root

resorption could be observed along the entire root surface(Fig. 5b).

The non-demineralized teeth showed similar pathological features to those of the demineralized teeth at the early stage of healing. However, at the later stage of 8 days after reimplantation, the inflammatory reactions remained at the cervical area due to the presence of a long crevice between the root surface and the connective tissue(Fig. 6). The gingival epithelium grew apically over the dentino-enamel junction, underneath which the infiltrated inflammatory cells remained(Fig. 6a). The connective tissue adjacent to the middle portion of the root was composed of fibroblasts in relatively low density(Fig. 6b). On the other hand, numerous fibroblasts were observed between the root surface and the alveolar bone, especially adjacent to the bone(Fig. 6c).

Ⅳ. DISCUSSION

1. Merits of small-sized animals:

One of the most important factors in animal experiment is how to standardize the experimental procedures and how to minimize the experimental variables. Individual differences between experimental animals has always been a concern. This is especially true when larger animals are used. It has been reported that the healing may vary not only as a result of uncontrolled experimental variables, but also due to the differences in biological responses between the animals or to fortuitous traumatic factors in the study of periodontal repair in dogs(Wikesjo 1991).

The two animal models currently most used are the monkey and the beagle dog. The major reasons for using these two animal models are first, it is easy to create periodontal disease similar to humans in these animals and second, their teeth are big enough to manage various periodontal and endodontic experiments. In the majority of models using these animals, periodontal disease has been experimentally induced with several features, such as traumatically-induced disease with ligatures or elastic bands, surgically-created disease with periodontal defects, or their combination, although some instances animals with naturally occurring periodontal disease have been used (Bogle G 1981, Aukhil I 1983, Bogle G 1983, Bogle G 1985). Even with these advantages, monkeys and dogs are very costly both to purchase and maintain. As well, their teeth are big and the supporting bone is rigid, which makes the extraction complicated.

In contrast, the purchase and maintenance of small animals, like rats, are fairly inexpensive. It follows that instead of being restricted to observing the stage of healing at a few selected points in time, investigators can include several animals at many different stages of healing. This allows for a sequential study of the processes involved. In addition, the use of a larger number of animals and experimental sites can be more powerful for statistical analyses. Besides, small-sized animals are easy to handle, and more importantly they allow the minimum amount of radioisotopes in the design of a given experimental protocol.

In general, the genetic consistency of the rat species is relatively well maintained compared with dogs and monkeys. This genetic consistency is thought to serve more homogenous experimental responses. Again, the large number of animals can compensate for any detrimental factors during the experiment, too.

The rapid tissue healing cycle of small animals also provides a good opportunity to observe the whole healing procedures in a relatively short time. Although the initial surface root resorption begins from as early as seven days after the injury (Andreasen 1980), the observation periods for larger animals usually require more time. In many of the monkey resorption studies, the animals were sacrificed at the 8th week after the root injury (Andreasen 1980a, Andreasen 1980b, Andreasen 1980c), while it was at the 20th day for dogs (Klinge 1984). In humans, the clinical observation time is even much longer, which is from 2 months up to several years (Andreasen 1966a, Andreasen 1966b). In contrast, rats (Hellsing 1993) and mice (Wesselink 1986, Wheeler 1993) have been reported to show distinct root resorption in between 10 and 14 days. In this study, the resorption began to appear as early as 6 days on the cementum surface(Fig 4b)

and then spread over the whole root surface by the 12th day(Fig. 5).

In this experiment, we could observe the serial events of periodontal healing after reimplantation. On the 1st day after reimplantation, the wound was filled primarily with coagulum. Clear demarcation could be made at the junction of the coagulum and the healthy periodontal fibroblasts in the PDL remnant attached to the alveolar bone surface(Fig. 1). On the 2nd day, the fibroblasts began to migrate actively toward the coagulum and root surface(Fig. 2). Healing took place faster in the apical region where the healing was composed of numerous PDL fibroblasts and collagen fibers. On the 4th day, the apical half showed relatively good healing, but the newly formed collagen fibers were alligned parallel to the root surface(Fig. 3). The cervical wound area still contained coagulum and showed the least progress in wound healing. The overall healing by the 6th day after reimplantation was much improved and was characterized by the presence of numerous fibroblasts and collagen fibers between the cells(Fig. 4). However, the fibroblasts in PDL still showed a parallel arrangement to the root surface, and the apical portion of the root had started to show the cementum resorption. On the 12th day, the root demonstrated extensive resorption along the entire root surface, except the cervical area(Fig. 5). Periodontal fibers still showed a parallel arrangement to the root surface.

2. Trauma methods:

Various injuries have been applied in order to develop root resorption. Heat injury (Atrizadeh 1971), which was applied through the root canal, produced initial necrosis of the periodontium at three days. Two weeks after the injury, resorption began to appear in the necrotized area, then increased progressively until 4 weeks.

Wesselink and associates (Wesselink 1986) traumatized mouse incisors by the local application of liquid nitrogen to the outer surface of the lower jaw. They observed the initial necrosis of periodontal ligament cells which was followed by the repopulation of cell-like fibroblasts and macrophages. From the 3rd day after the trauma, mineral crystallites were deposited along the cementum covering the lingual, mesial, and lateral surfaces of the incisor, finally resulting in a 4 - 6 micron thick layer. During the period of 7 - 12 days following cold application, the layer of mineralized material started to be phagocytosed and degraded, which led to extensive root resorption and ankylosis. Later, a cryoprobe technique was introduced (Wheeler 1993, Ng 1990) in order to localize the cold trauma to the mouse incisor. Resorption began to appear at the 7th day and gradually increased in size and number until the 10th day. No further noticeable increase was observed thereafter. Although cold injury provides a simple trauma to develop root resorption and ankylosis, a couple of problems still remain. One is that freezing trauma does not have the same characteristics as routine avulsion trauma. The second is that with this experimental setup, it would not be possible to apply the resorption preventing factors directly to the root surface, although the systemic application is still possible.

From a clinical point of view, re-implantaion after root injury might be the most suitable method for root resorption studies. Hellsing and Hammarstrom (Hellsing 1993) performed reimplantation of rat molar teeth after devitalizing the attached periodontal ligament. What they basically wanted was to see whether the re-implanted teeth could create consistent root resorptions. In an effort to remove the viable periodontal tissue quickly, they used Dakin's solution, which is a kind of house detergent. Induction was most reproducible with Dakin's solution after keeping the rats on a liquid diet for up to 3 weeks after reimplantation, although the devitalizing technique did not show any morphological differences.

3. Less injury during extraction:

In order to perform a tooth reimplantation study, it is of utmost importantance to extract the tooth without noticiable injury to the tooth itself and the surrounding tissues. According to our personal experience, it has not been easy to extract molar teeth from dogs and cats. In most cases, the tooth had to

be split in half, mesial and distal, for safe extraction. This is same in rats, too. The older the rat is, the more likely the roots are to fracture. In rats, any teeth older than three months are prone to break, and are not recommended for this purpose (Hellsing 1993). Here we used 30(plus minus 5days)day old rats. The 30-day-old rat used in this experiment, showed all five fully-developed roots with a considerable amount of cellular cementum apposited at the apical third. This observation was a little faster than that of previous findings (Schour 1949), where the secondary cementum begins to form about the 35th day. Although the apical foramens were not yet completely closed, the thickness of the root wall seemed to be strong enough to withstand the extraction force. Fracture of the roots was rarely observed. It seems that this period of root development is appropriate for extraction and reimplantation because the surrounding bone is not fully demineralized, which makes extraction easier. This also suggests that the surrounding periodontium is still in an actively developing state, which will lead to an active healing sequence not only in periodontium, but also in pulpal regeneration studies (Kvinnsland 1991, Byers 1992).

4. β-APN:

β-APN is known to reduce the tensile strength of PDL collagen (Borstein 1970, Fry 1962) and allowed gentler extraction of the teeth. This can minimize damage to the alveolar bone and the surrounding soft tissues. β-APN interferes with an amine oxidase, thereby preventing the formation of lysyl-derived aldehyde groups on the collagen alpha chains (Borstein 1970). Because of the consequent reduction of reactive aldehyde groups, the collagen molecules fail to become cross-linked and the tensile strength of the collagen fibers is greatly reduced (Fry 1962). The pathologic effect of this molecular defect is most pronounced in connective tissue exposed to increased stress such as in articulations, sites of muscle insertions, and in the periodontal ligament of functional teeth (Gardner 1958, Ponseti 1954, Krikos 1959, Barrington 1966, Sciaky 1961, Levene 1973). Previous studies (Cho 1984a, Cho 1984b) showed palisading of the fibroblasts in β-APN treated mouse periodontium.

With β-APN pre-treatment, most of the first molar teeth could be extracted intact with all 5 roots. This untraumatic extraction is very important because the peridontium receives various degrees of trauma when it is extracted. This trauma produces different healing consequences. In a topographic study of surface and inflammatory resorption after replantation of mature permanent incisors in monkeys, Andreasen (Andreasen 1980a) correlated the degree of root resorption with extraction time. The longer the extraction time, the more root resorption could be observed. He also found the direction of extraction force influenced the onset of resorption (Andreasen 1980b, Andreasen 1980c). He postulated that the bulky area of the root received more compression in contrast to the flat or concave area, which led to more root resorption. The root surface facing the alveolar bone showed more resorption than the supra-alveolar area, indicating that the infra-alveolar area might have received more injury. Therefore, the minimally stressed extraction method is very important for obtaining consistent results.

Our previous study (Lin 1994) revealed that this extraction method left a band of PDL of homogeneous width along the alveolar bone surface and created a condition in which PDL fibers were the major cell type in the socket. The majority of fibers in the remaining PDL were reported to retain their viability and their ability to proliferate, except for the small number of damaged fibers at the margin of the torn PDL.

5. Rationale for non-autogenous transplantation:

We reimplanted the tooth which was obtained from the preceding animal. This provided enough pretreatment time before reimplantation. After extraction, the teeth were incubated for 3 - 4 hours at 37C in 0.02M Tris-HCl, pH 7.4 containing 800 units/ml of bacterial collagenase(Calbiochem, Collagenase, Clostridium histiolyticum, TypeII), 0.2M NaCl and 50mM CaCl2 to denude the root surface of the remnants of periodontal ligament. This was originally to simulate clinical conditions of periodontal disease. Prepared teeth were stored in a refrigerator and used

for the next animal. A question related to the cross-immunity of two different individuals may be raised. Although this possibility cannot be totally ruled out, it is not likely to happen for these hard tissues within such a short time period.

6. Effect of remaining pulp:

A question as to whether the unsealed remaining pulp space in a rat would affect the periodontal healing was raised (Hellsing 1993). Previous studies of induced ankylosis in dogs and monkeys also recommended endodontic treatment within the first few weeks after reimplantation to prevent inflammatory resorption (Cvek 1974, Andreasen 1981). However, completing a root canal treatment in rat molar is technically impossible. Hellsing and Hammarstrom (Hellsing 1993) used Dakin's solution primarily to remove the remaining periodontal tissues from the extracted tooth. However, he postulated that the bactericidal effect of the Dakin's solution might have helped prevent an inflammatory reaction in the empty pulp space. In this experiment, we sealed the apical foramens of all five roots under the microscope. For the mesio-buccal root(the largest root of the first molar) especially, the marginal wax interfacing of the inner surface of the root was melted using a sharp, heated instrument to secure a better seal. Some small roots like the middle buccal root were left unsealed because of the technical difficulty. From this animal model, no delineable signs of inflammatory resorption could be observed.

7. Problems:

An appreciable aspect of an animal model to study periodontal healing lies in whether it can recreate as accurately as possible the existing conditions accompanying periodontal diseases in humans. Reimplantation, as used in our model, occurs in a healthy socket unaffected by previous or progressing periodontal desease. Many of the symptoms classically present as a result of periodontal disease are not found here. For example, there was no reduction in the height of the alveolar crest. In order to simulate the condition of the root surfaces of periodontally involved teeth, the periodontal ligament remaining after the extraction, was digested using bacterial collagenase. However, the tissues surrounding the reimplantation site has never been involved in any chronic pathological process. This does not eliminate the possibility that the other local differences may exist in the environment surrounding these reimplanted teeth versus teeth naturally involved in a periodontal lesion. Also, animals have no known susceptibility to destructive periodontal disease. It is difficult to recognize how these issues may affect the conditions in which healing will take place. The use of a dog or monkey model with either natural or induced periodontal disease is probably preferable in this regard, although some limitations exist there, too, due for instance to the fact of having to use older animals in the case of naturally occurring periodontitis.

This model seems to be adequate particularly for the resorption study simulating traumatically avulsed teeth. As was shown in Fig. 4 and Fig. 5, distinctive cementoclasts and dentinoclasts were observed from the 6th day after reimplantation and gradually increased thereafter. Various resorption inhibiting factors can easily be tested. Another limitation of this tooth model is related to the small size of the animal and consequently the oral cavity. This precludes the use of this animal model to study certain treatment modalities, for example, GTR or to measure changes in classically-used clinical parameters. In addtion, it is difficult to control the repositioning of the teeth in their socket. These may result in some variables in the position of the alveolar crest and the attached gingiva.

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PICTURES

Fig. 1. Photomicrographs showing the wound areas 1 day after reimplantation of a demineralized molar.
The wound area is demarcated with dotted lines. Note the presence of coagulum between gingival connective tissue and the root surface shown in the area of (a). Also, note the healthy PDL fibroblasts(arrow heads) in the PDL remnant attached to the alveolar bone and a few PDL fibroblasts (open arrows) migrating toward the wound area(b).
AB; alveolar bone
BV; blood vessel
PDL; periodontal ligament
C; coagulum
I; inflammatory cells
GE; gingival epithelium
GC; gingival connective tissue
DEJ; dentino-enamel junction
D; dentin
CMSC; cellular mixed stratified cementum
EFC; extrinsic fiber cementum

Fig. 2. Photomicrographs demonstrating the healing of periodontal tissues 2 days after reimplantation of demineralized molars.
The wound space is primarily filled with coagulum rich in fibrin. Note the presence of intact gingival connective tissue(a), and fibroblasts(arrow heads) migrating actively toward coagulum(b), the alveolar bone(c), and the root surface(d). In the area of (e), PDL fibroblasts also migrate into the wound area. The apical portion of the PDL is healed and is composed of numerous PDL fibroblasts and collagen fibers(f).
AB; alveolar bone
PDL; periodontal ligament
C; cementum
GE; gingival epithelium
GC; gingival connective tissue
D; dentin

Fig. 3. Photomicrographs revealing the healing of periodontal tissues 4 days after reimplantation of demineralized molars.
The lower half of the PDL is relatively well healed. Even though numerous fibroblasts(arrows) and newly formed collagen fibers(arrow heads) are observed in this area, they demonstrate a parallel arrangement to the root surface. The upper portion of the PDL(b) shows many fibroblasts(arrow) filling in the wound area between the alveolar bone and the gingival connective tissue. The cervical wound area still contains coagulum and shows the least progress in wound healing(a). Note the infiltrating fibroblasts(arrow heads) and fibrin(arrow).
AB; alveolar bone
C; cementum
D; dentin
GE; gingival epithelium
GC; gingival connective tissue
DEJ; dentino-enamel junction

Fig. 4. Photomicrographs showing periodontal regeneration 6 days after reimplantation of demineralized molars. The overall healing of the periodontal ligament is improved and characterized by the presence of numerous fibroblasts and collagen fibers between the cells. However, the fibroblasts in PDL still show a parallel arrangement to the root surface, and the apical portion of the root demonstrates extensive root resorption(b). Note the improvement in healing of the gingival connective tissue(a).
AB; alveolar bone
C; cementum
D; dentin
GE; gingival epithelium
GC; gingival connective tissue
CC; cementoclasts

Fig. 5. Photomicrographs showing periodontal regeration 12 days after reimplantation of a demineralized molar. Note the improved healing in the apical wound area, and the complete healing in the area between the buccal side of the alveolar bone surface and the gingival connective tissue(a), and in the PDL(b). However, the fibroblasts in PDL still show a parallel arrangement to the root surface, and the apical portion of the root starts to demonstrate root resorption(b).
JE; junctional epithelium
DC; dentinoclasts

Fig. 6. Photomicrographs demonstrating a healing process 8 days after reimplantation of an non-demineralized molar.
Even though healing of the gingival connective tissue(a,b) and the PDL(c) is nearly completed, an artificial split(arrow heads) between the root surface and the connective tissue is prominent.
GE; gingival epithelium
GC; gingival connective tissue

사진부도 ①

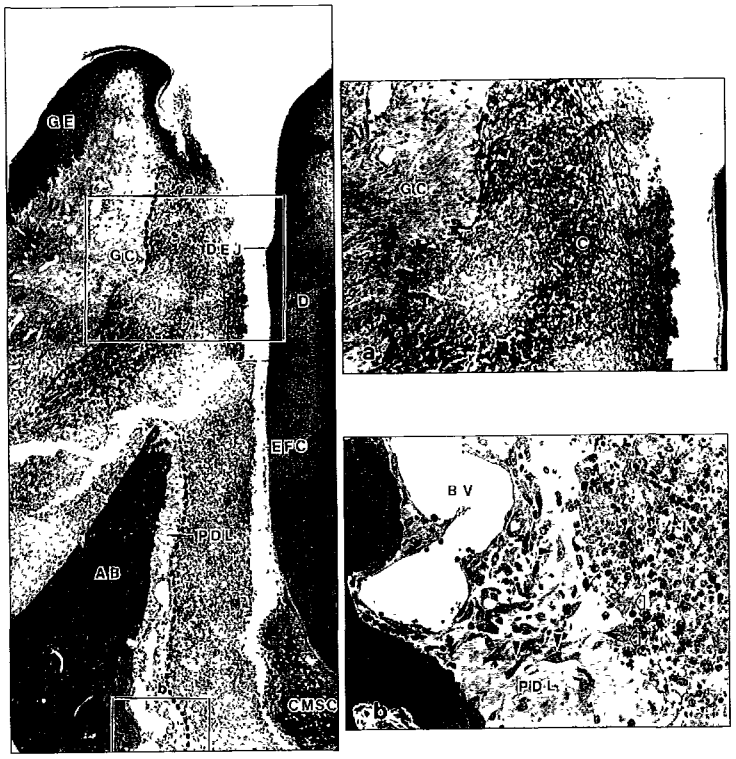

Fig. 1

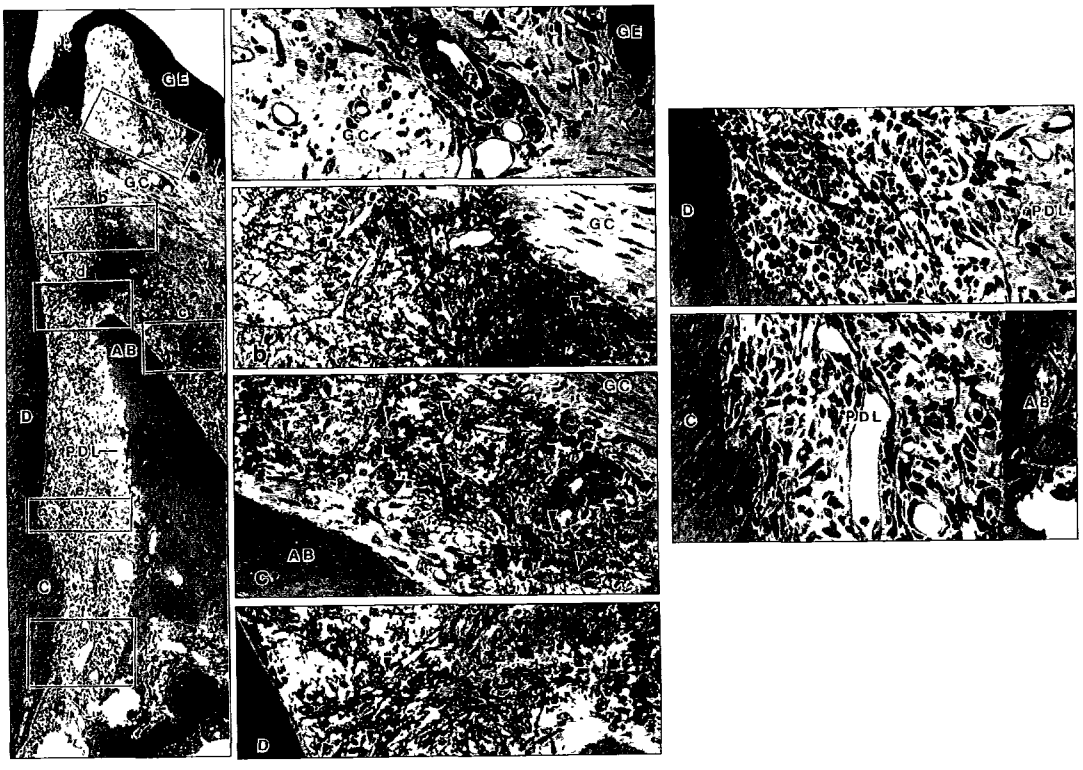

Fig. 2

사진부도 ②

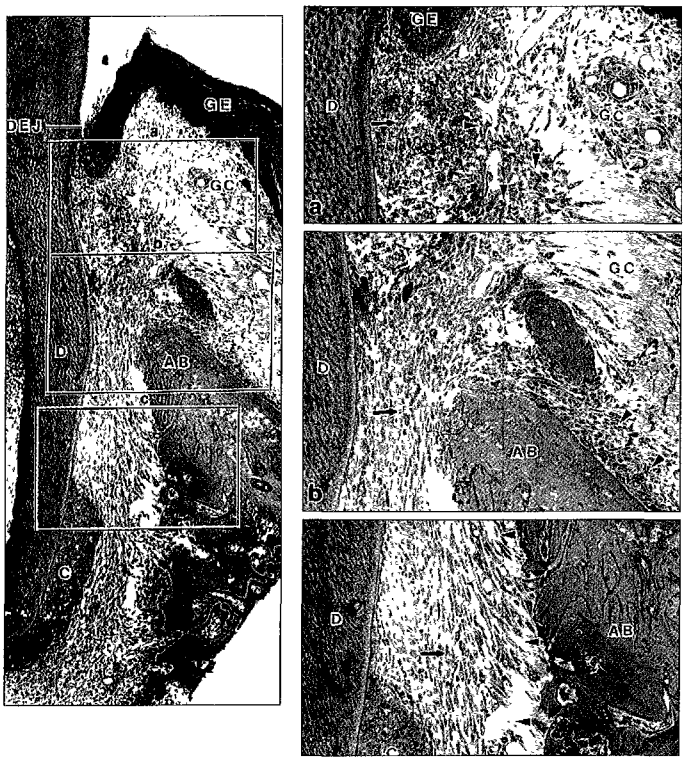

Fig. 3

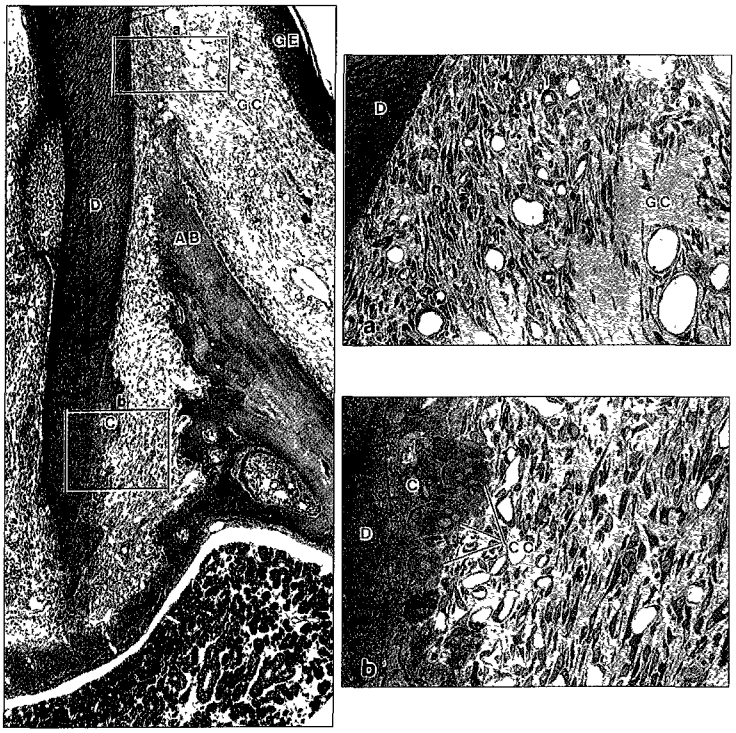

Fig. 4

사진부도 ③

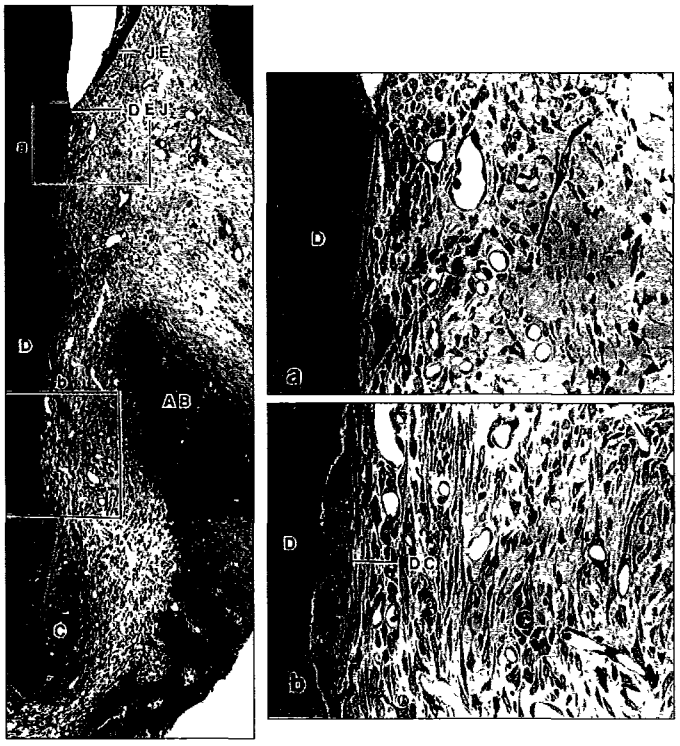

Fig. 5

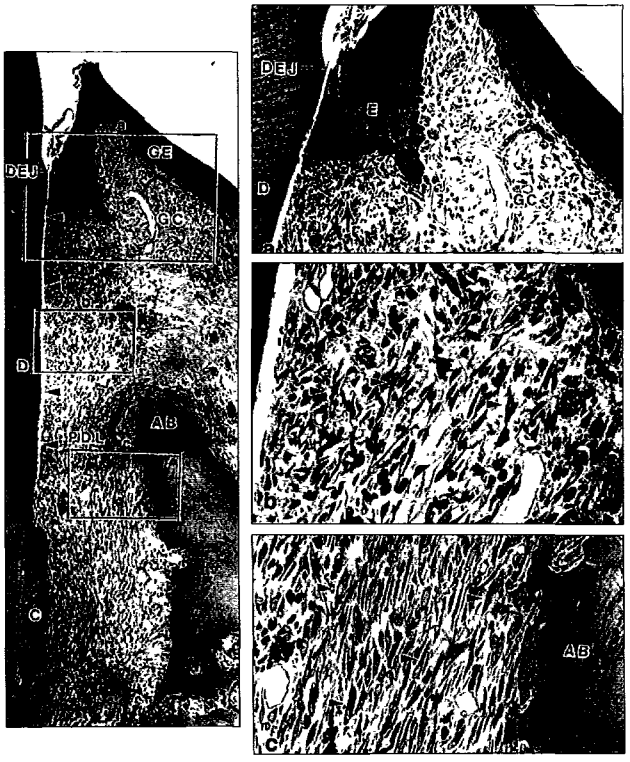

Fig. 6