Histologic evaluation of root-surface healing after root contact or approximation during placement of mini-implants

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Introduction: Placement of mini-implants carries with it the risk of iatrogenic damage to the adjacent root surfaces. The aims of this study were to assess the type of trauma incurred on tooth roots after contact or approximation with mini-implants during placement, and to observe and analyze the healing responses via histologic analysis. Methods: Four male minipigs were used as experimental subjects. Twenty mini-implants (1.6 × 8 mm) were implanted in each minipig into the buccal sides of all 4 quadrants between the roots of teeth so that contact or approximation between the mini-implant and root surface occurred, with the aid of dental fluoroscopy. All mini-implants on the left side of the mouth were left in situ, and all mini-implants on the right side were removed immediately after placement. The minipigs were killed at 4-week intervals up to week 16, and histologic sections were made. Results: When mini-implants were left in situ, the root surface was mostly resorbed away from the mini-implant thread. Partial repair started at 8 weeks. When the mini-implant thread was left touching the root, there was no normal healing response. If the mini-implant was placed less than 1 mm from the periodontal ligament, resorption was evident on the root surface. Abnormal healing responses were seen when the pulp tissue was ruptured, mostly through osteodentin formation. In all instances after mini-implant removal immediately after placement, varying degrees of cementum repair was observed. Conclusions: Immediate removal of the mini-implant leads to cementum repair, whereas leaving the mini-implant in place will cause either a delay in repair or no repair. Placing mini-implants less than 1 mm from the root surface causes root-surface resorption. (Am J Orthod Dentofacial Orthop 2011;139:752-60)

Mini-implants are now being extensively used in orthodontic treatment. They have opened up a new field of possibilities for treatment that was nearly impossible before the concept of absolute anchorage became available to us in the form of mini-implants. The small size and ease of placement have made mini-implants user-friendly; they are also relatively comfortable for the patient.¹ The main concerns with mini-implants are the failure rate and potential trauma to the surrounding structures and the consequences of the damage. Potential complications of root injury mentioned in the literature include loss of tooth vitality, osteosclerosis, and dentoalveolar ankylosis.²-⁴ Iatrogenic root damage from mini-implants is an important aspect but has had little attention until recently. Fabbroni et al⁵ investigated root contact after transalveolar screw placement for mandibular fractures in a clinical study. Although some implant and root contact occurred, they concluded that the incidence of clinically significant damage appeared to be low. Ascherickx et al² found that, after accidental damage to tooth roots in beagles, almost complete repair of the cementum occurred in 18 weeks and started at the earliest 12 weeks after the screw was removed. In a recent study by Kadioglu et al⁶, scanning electron microscopy was used to observe root surfaces after intentional contact between root and mini-implant. They found that most of the repair of root surfaces occurred 8 weeks after removal of the screw or the orthodontic force. Scientific evidence regarding these side effects, especially histologic evaluation of root surfaces, is still lacking in the literature.

Although mini-implants can be placed in various areas of the mouth, the interradicular spaces between adjacent teeth are the most common sites. These sites

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have an increased risk of causing iatrogenic root damage and require careful planning and consideration before mini-implant placement. In a recent study with computed tomography images, the greatest amount of mesiodistal bone between adjacent roots buccally was 3.5 mm between the first and second premolars. There is also evidence that mini-implants migrate up to 0.5 mm during treatment. This must be considered when choosing sites for placement. Liou et al stated that the minimum distance between the root surface and the mini-implant should be 2 mm to prevent root damage. Maino et al, on the other hand, recommended that a 1-mm clearance between mini-implant and root is sufficient to maintain periodontal health and mini-implant stability.

The extent of root damage from mini-implant contact can be well ascertained from histologic analysis. It is of special interest to observe the different types of responses when the mini-implant is left touching the root surface and when the stimulus has been removed. The aims of this study were to assess the type of trauma incurred on tooth roots after contact or approximation of mini-implants during placement and to observe and analyze the healing responses via histologic analysis.

MATERIAL AND METHODS

Four male minipigs (specific pathogen-free micro-pigs) weighing between 31 and 43 kg and aged over 12 months were used as the experimental subjects. A total of 20 mini-implants (16-JA-008H, JA type, Jeil Medical, Seoul, Korea) measuring 1.6 mm in diameter and 8 mm in length were placed in each minipig (Fig 1).

After administration of intramuscular preanesthetic medication, anesthesia was maintained through inhalation of isoflurane (2.0%, Terell isoflurane, Minrad, Orchard Park, NY). The mini-implants were placed into the buccal sides of all 4 quadrants, between the roots of teeth so that contact or close approximation between the mini-implant and root surface occurred (Fig 2). The mini-implants were placed without making incisions in the mucosa and without a separate drilling procedure. If it was uncertain whether contact with the root surface were made or if adequate root approximation was unsuccessful due to thick mucosa, the mini-implants were excluded. To aid in visualizing the roots and the mini-implants, fluoroscopy was used during placement. Fluoroscopy is an imaging technique that uses an x-ray source passed to a monitor to view real-time images of moving structures (40F, Dreamray, Busan, Korea). Lead vests were worn, and exposure times were kept to a minimum. After placement of all mini-implants, 4 tattoo marks were made around each mini-implant in the maxilla and the mandible of the right side. The mini-implants on the right side were removed immediately after placement.

Three fluorochromatic dyes (Sigma, St Louis, Mo) were injected intramuscularly for fluorescent microscopy investigation. Tetracycline hydrogen chloride was administered (15 mg/kg) at 2 weeks and 14 weeks after placement, calcein (10 mg/kg) at 6 weeks after placement, and alizarin red (30 mg/kg) at 10 weeks after placement. The minipigs were killed one at a time at 4-week intervals, for a total experimental period of 16 weeks. After killing each minipig, the specimens of each mini-implant on the left side, and the areas where
the mini-implants were removed on the right side, were prepared in the axial plane by using a cutting and grinding system (Exakt Apparatebau, Nordstedt, Germany) according to the method reported by Donath and Breu-

ner.10 The thickness of the specimens was about 40 to 50 μm. The specimens were stained with hematoxylin and eosin. Each specimen was sectioned so that the contact area between the mini-implant and the root surface was well represented.

RESULTS

Of the total number of mini-implants placed, only the specimens that best represented the contact area between root and implant were analyzed. This amounted to an average of 7 sections per animal for the mini-implants in-situ group, and 3 sections per animal for the mini-implant removed group. The small number of slides meant that statistical analysis was difficult to carry out. Only observational analysis was undertaken.

The observed results were separated into 2 groups: healing of the root surface when the mini-implants were left in situ, and changes in the root surface and surrounding structures after the mini-implants were removed.

A summary of the root healing observed when the mini-implants were left in situ is shown in the Table. The root-contact group was divided into 2 subgroups, the first containing those that made contact or were in close proximity with the dentin or the periodontal ligament (PDL), and the second subgroup included those that penetrated into the pulp. Overall, no teeth showed signs of ankylosis after contact with the mini-implant. A patent PDL space was maintained for all observed specimens throughout the 16-week experimental period. The samples in the root-contact group were further classified into 3 types, and the pulp penetration group into 2 types, according to the type of healing observed.

The root-contact only group included 3 types.

1. Resorption of the root in proximity. Even though direct contact between the root and the implant was not made, resorption of the root surface was observed. The distance between the implant and the root surface was less than 1 mm. Even though there was bone between the implant and the root surface, resorption still occurred on the root surface (Fig 3).

2. Resorption away from the thread. After contact of the implant with the root surface and with time, resorption occurred away from the thread of the mini-implant. There was no bone between the thread and the root surface, just soft tissue resembling the PDL (Fig 4).

3. Thread touching the root. After contact of the mini-implant thread with the root surface, it stayed in contact with no resorption of the root surface (Fig 5).

The penetration through the pulp included 2 types.

1. Dentin-tissue rupture only. No healing response was observed.

2. Dentin-tissue rupture, with osteodentin. There was rapid formation of irregular reparative dentin with cellular inclusions (Fig 6).

Resorption of the root in proximity was seen in specimens at 4 and 8 weeks. A small area of cementum healing was observed in 1 area of the specimen at 4 weeks with evidence of rapid resorption and repair (Fig 3). The 8-week specimen showed no obvious signs of cementum deposition on the section stained with hematoxylin and eosin, but it did begin to indicate some hard-tissue formation through calcein deposition from week 6 on fluorescent microscopy.

For the sections that showed root resorption away from the thread, varying degrees of repair were seen. At 4 weeks, no sections showed a healing response, only resorption lacunae following the shape of the implant threads. On higher magnification, however, osteoblasts were seen arranged in a row in the PDL layer, ready to begin the repair process. By week 8, partial repair of the cementum was seen in 1 specimen of 3. At 12 weeks, 3 of 4 specimens showed cementum repair on the damaged root surface (Fig 4).

When any part of the mini-implant thread was left touching the root surface, it was interesting to see that, during the entire observation period, no definite cementum repair was observed on the damaged root surface (Fig 5). In 1 specimen of the 16-week group, an abnormal overproduction of cementum was seen around the thread of the implant.

The healing responses of the pulp-penetration group were mostly through osteodentin formation (Fig 6). This was observed as early as week 4 and up to week 16. The

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<tr>
<th>Specimens (n)</th>
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<tr>
<td>Root contact only</td>
<td>Resorption of root in proximity</td>
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<td>Root contact only</td>
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<td>Root contact only</td>
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<tr>
<td>Penetration through pulp</td>
<td>Dentin tissue rupture only</td>
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<td>Penetration through pulp</td>
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dentin-tissue rupture only type was observed in the 4-week specimens only.

Healing in the group of specimens with the mini-implants removed showed that, in most cases, cementum repair occurred on the damaged root surface. At week 4, there was partial repair with cementum formation in some areas only. From weeks 8 to 16, all sections showed cementum repair (Fig 7). At week 16, a specimen with dentin and pulp penetration damage demonstrated osteodentin formation, with maintenance of the PDL (Fig 8).

**DISCUSSION**

Injury refers to the disruption of the continuity of tissues, and healing is the reestablishment of that...
continuity. The healing responses of the root—PDL, cementum, pulp, and dentin—after injury from mini-implant placement were examined in this study. An interesting finding was the occurrence of surface root resorption caused indirectly by the mini-implant close to the root (Fig 3). Even though there was a clear width of bone of about 1 mm between the root surface and the mini-implant, evidence of root resorption was seen. Pressure from placement of the mini-implant through the thin layer of alveolar bone might have had an effect on the root surface. Pressure from the implant will cause compression of the PDL, and this compression can cause indirect damage to the root surface. It has been shown that drying of the PDL, physical removal from the root surface, and compression of the PDL are injuries that can cause root resorption. After injury of the PDL, wound healing begins when damaged tissue is removed by macrophage or osteoclastic activity. During this activity, not only are necrotic PDL tissue remnants removed, but also sometimes bone and

Fig 5. Thread touching the root (specimen from minipig killed at 16 weeks). A, Lodging of the mini-implant between adjacent roots with severe force means that the threads of the implant stay in contact with the root surface. The increased force during placement caused slight bending of the mini-implant. B, Higher magnification showing the point of contact between the implant and the root surface (short red arrows). There is no evidence of cementum repair. P, Pulp; D, dentin; M, mini-implant.

Fig 6. Root penetration of mini-implant (specimen from minipig killed at 4 weeks). A, Dentin tissue rupture with osteodentin formation. The area in the red box is shown in B. B, Higher magnification showing rapid formation of irregular reparative dentin (short yellow arrows) with cellular inclusions (long red arrows). P, Pulp; D, dentin; PDL, periodontal ligament; M, mini-implant; B, bone.
cementum. Injuries that can elicit wound-healing responses include hemorrhage, edema, rupture, and contusion of the PDL. Pressure from bleeding into the PDL might have also elicited minor areas of damage on the root surface. When there is no relief of pressure from bleeding, it can cause surface resorption. An explanation for this phenomenon is that a higher frequency of surface resorption is seen in dental concussion rather than subluxation injuries. It has been shown that this kind of edema directly affects the arrangement and structure of the extracellular matrix of the PDL. Faltin et al. observing hyalinization after tooth intrusion, stated that mechanical stress might be responsible for the vascular flow alteration that triggers cellular degeneration, leading to hyalinization. Removal of this hyalinized necrotic tissue—in other words, remodeling of the PDL layer—can be said to be the beginning of root resorption.

Fig 7. Mini-implant removed after root contact (specimen from minipig killed at 12 weeks). A, Resorption of root surface was observed. The area in the red box is shown in B and C. B, Higher magnification showing cellular cementum repair on the damaged root surface (short black arrows). C, Fluorescent microscopy examination shows repair through tetracycline (yellow arrow), calcein (green arrow), and alizarin red (orange arrow) deposition on root surface. D, Dentin; PDL, periodontal ligament; B, bone.

Fig 8. Mini-implant removed after dentin rupture (specimen from minipig killed at 16 weeks). A, PDL space has been maintained even after dentin rupture and repair. The area in the red box is shown in B and C. B, Irregular osteodentin includes cellular inclusions (short yellow arrows). C, Fluorescent microscopy examination shows repair of damaged dentin through tetracycline (long yellow arrows) and calcein (short green arrows) deposition. P, Pulp; D, dentin; PDL, periodontal ligament; B, bone.
The diameter of the mini-implants used in this study was 1.6 mm. Clinically, any implant diameter greater than this might increase the pressure exerted on the PDL. Care should be taken when placing larger mini-implants interradicularly, because they could increase the chances of root resorption. It seems possible also that predrilling before mini-implant placement might decrease the pressure exerted on the root surface. However, further clinical and histologic studies are required.

For dental implant placement, it has been suggested that a 3-mm clearance should be given between the implant and an adjacent natural tooth. For mini-implants, Liou et al recommended that at least a 2-mm space is required between the mini-implant and the root, because the mini-implant was seen to migrate between −1.0 and 1.5 mm during orthodontic loading. Other studies have measured the distance between roots by using radiographic or computed tomography images to offer guidelines for the most effective sites for mini-implant placement causing the least amount of root damage. In a study where the distances between roots were measured on volumetric tomographic images, the authors suggested that at least 3.5 mm of bone is needed as a safe zone in the interradicular space for mini-implants with a diameter of 1.5 mm. This was with the assumption that a minimum clearance of 1 mm of alveolar bone is sufficient for periodontal health. Huang et al recommended at least a 1.5-mm clearance between the mini-implant and the root surface. Our results suggest that, when the clearance of bone between root and implant is less than 1 mm, surface resorption takes place. In addition, when the mini-implant ruptured through dentin and pulp tissue, the dentin responded by rapidly depositing tertiary dentin with sparse and irregular tubular patterns with cellular inclusions known as osteodontin (Figs 6 and 8), which has a similar appearance to bone. Rupturing the pulp tissue leads to irreversible consequences and should be prevented when possible. In contrast, in all instances when the mini-implants were removed immediately after placement, cementum repair occurred on the damaged root surfaces. As long as continuous contact with the root is avoided, any damage was seen to be repairable. Taking into account such factors, it is important to ensure that mini-implants are accurately placed between the roots. Presurgical periapical radiographs are essential, and postsurgical radiographs are also recommended to check their correct placement. If resistance is felt during placement, or the patient responds with pain, the mini-implant should be removed and replaced. It has been suggested that a patient knows when the mini-implant touches the PDL, as long as the local anesthetic is limited to a small amount in the soft tissues. Various guides and stents are available to aid in the correct placement of mini-implants. Although the use of guides and stents might be time-consuming and somewhat cumbersome, they will invariably help to prevent root damage. Other methods of preventing root damage include placing the mini-implant at approximately 20° or 30° to the long axis of the tooth.

Kuroda et al suggested that the proximity of mini-implants to the adjacent tooth root is a major risk factor for the failure of mini-implants. Although failure of mini-implants was not assessed in this study, contact or close proximity of mini-implants to the root might increase the failure rate. It was suggested that occlusal forces are transferred to the mini-implant via the root, and this leads to mobility. Asscherickx et al concluded that the distance from the mini-implant to the marginal ridge is an important factor for success. There is a higher rate of success for mini-implants placed at least 1 mm from the marginal ridge.

With root resorption after orthodontic tooth movement, histologic repair has been observed as early as 1 week after cessation of orthodontic forces. The characteristics of root resorption from iatrogenic trauma, such as that resulting from mini-implant contact, might differ from that resulting from orthodontic force. The healing responses can also differ. The healing process for orthodontically moved roots has been shown to increase over the first 4 weeks and slow down to a steady phase after 5 to 6 weeks of retention. In our specimens, the characteristics of repair varied according to the varying conditions. When repair did occur, it showed a gradually increasing pattern, with partial repair at 4 weeks, increasing to full repair of many sections up to week 16. Considering the conditions required for commencement of root repair after damage, we noted that, when the mini-implant was left contacting the root surface, there was minimal healing in most of the specimens. In contrast, as long as the root surface resorbed away from the implant thread, some cementum deposition occurred. Maino et al found that, when the tooth was pushed against the mini-implants with orthodontic force, there was no sign of repair in the resorption lacunae. However, when root contact was discontinued, abundant cellular cementum was deposited. Kadioglu et al also concluded that swift repair of tooth surfaces occurred once the mini-implant or the orthodontic force was removed. If the tooth is moved into contact with the mini-implant, it could be hypothesized that repair will not begin until the orthodontic force is removed. Although no orthodontic force was applied in our study, the results were similar. The histologic slides showed that, when the mini-implant was drilled between 2 roots...
with severe force, the mini-implant became lodged between the adjacent roots (Fig 5). Figure 5, A, shows that the high force with which the mini-implant was placed caused slight bending of the implant. This absence of mobility means that pressure between root and implant is maintained. Relief of pressure might be required to allow repair via cementum deposition. If the cementum is mechanically damaged, whether through trauma such as from mini-implant iatrogenic damage or orthodontic tooth movement, multinucleated cells will arrive near the surface to initiate resorption. Resorption is an active process that requires viable cells near the root surface. The first signs of root resorption have been defined as penetration of cells from the PDL into the mineralized cementum. The PDL has a high regenerative potential and plays a large role in the healing potential of damaged root surfaces. When a portion of the PDL is damaged, the viable PDL cells from adjacent areas have been reported to migrate into the damaged areas and provide reparative cells. Therefore, since root resorption is an active process requiring viable cells, severe trauma to the surrounding structures such as shown in this study or the absence of active PDL cells in the vicinity will prevent root resorption. Resorbing cells have been shown to require continuous stimulation during phagocytosis, and, without further stimulation, the process stops spontaneously.

Previous studies have indicated that physical removal of the PDL might also lead to extensive ankylosis after tooth replantation. In our study, there was no evidence of ankylosis. The PDL space was maintained in all sections throughout the experiment. In an experiment in which different areas of the PDL were removed by drying before tooth replantation, it was shown that ankylosis reaches its peak after 2 weeks and diminishes significantly by 4 to 8 weeks after replantation. Also, removal of 1 or 4 \( \text{mm}^2 \) of the PDL resulted in transient replacement resorption, whereas the removal of 9 and 16 \( \text{mm}^2 \) resulted in permanent ankylosis. Andresen and Kristerson found that up to a 2-mm width of PDL loss on the root surface can be repaired by new attachment without ankylosis. Although these studies deal with resorption after tooth replantation, it gives us some indication of the healing responses of the PDL. Minor areas of damage will be repaired from moderate amounts of surviving cells in the adjacent intact PDL. Chen et al found that, after intentional root contact, the PDL space was well maintained with no ankylosytic spots. In our experiment, the first histologic samples were taken 4 weeks after mini-implant placement; therefore, it could be assumed that any ankylosis might have been resorbed by viable cells in the adjacent PDL.

An important aspect of the study is that, even though root healing was observed over a 16-week period, each 4-week period was represented by a separate animal. It is inevitable that there were some differences between animals. However, we attempted to standardize the conditions of the 4 animals regarding age, sex, weight, and maintenance. Future studies involving measurement of bone-implant contact and its statistical significance in association with mini-implant root contact will be helpful.

CONCLUSIONS

If the mini-implant has made contact with the root surface, immediate removal and replacement is suggested. If, however, the mini-implant is left in place, varying responses can be expected. When the root resorbs away from the mini-implant thread, cementum healing occurs in most instances after 12 weeks. When the mini-implant thread is left in contact with the root surface, mostly due to high force and severe trauma to the root during mini-implant placement, no healing occurs. When the conditions are not optimal, resorption and repair do not occur. The damage is irreversible when the mini-implant ruptures through thicker areas of dentin and into pulp tissue. An interesting finding from this study was that, when the mini-implant was placed less than 1 mm from the PDL, external root resorption occurred. Although no direct contact was made and there was bone between the implant and the root, resorption still occurred. It is recommended that at least a 1-mm space should be left between the mini-implant and the root surface during placement of mini-implants.

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