Histomorphometric analysis of different latency periods effect on new bone obtained by periosteal distraction: an experimental study in the rabbit model

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Objectives. The aim of this study was to evaluate the effects of latency period on the bone formation after periosteal distraction. For this purpose, a rabbit model was developed and histologic and histomorphometric analyses were conducted.

Study design. Periosteal distractors were custom designed and built from stainless steel. Rabbits were divided into 2 groups of 18 each according to the duration of latency period. In group 1, the latency period was 7 days, and in group 2 it was 1 day. Distraction was performed by activating the distractor 0.25 mm twice per day. A periosteal distraction of 7.0 mm was achieved after a distraction period of 10 days. Both groups were divided to 3 subgroups according to the rabbits being killed on the 15th, 30th, or 60th day of the consolidation period. Histologic and histomorphometric analyses were performed to evaluate the bone formation.

Results. In the histologic evaluation, new bone formation was observed on the lateral side of the mandible of all the rabbits. Histomorphometric measurements revealed that the mean area of newly formed bone formation was 2.62 cm² in group 1 and 3.26 cm² in group 2.

Conclusions. Periosteal distraction osteogenesis is a viable method in acquired and congenital alveolar ridge defects, resulting in new bone formation. Newly formed bone can be obtained by periosteal distraction osteogenesis applying different latency periods. (Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2011;111:539-546)

The treatment of patients who are partially or totally atrophically edentulous requires an ideal alveolar ridge that has adequate bone height and width. Common etiologies of alveolar bone loss are extraction, traumatic avulsion of teeth, periodontal disease, tumor resection, infections, and congenital deformity.1,2

Multiple treatment methods have been applied to augment the alveolar ridge. Current reconstructive alternatives for the atrophic bone may be grafting,3-6 guided bone regeneration (GBR),7,8 and alveolar distraction osteogenesis.1,2,9-13 Donor site morbidity and some graft resorption have limited the current use of bone grafting.14,15 Unfortunately, GBR is useful only for limited defects of the alveolar ridge.1

Successful results have been reported for the clinical application of distraction osteogenesis to maxillofacial bones.16-34 Briefly, distraction osteogenesis consists of 5 sequential periods: osteotomy, latency, distraction, consolidation, and remodeling. Latency is the period from bone division to the onset of traction and represents the time required for reparative callus formation between the osteotomized bone segments.35
Toth demonstrated the first application of alveolar ridge augmentation in humans. Gaggl et al. reported a new operative technique for alveolar ridge augmentation using a distraction implant.

Periosteum plays an active role in distraction osteogenesis via a highly vascularized internal region called the osteoblastic layer. This layer is composed of mesenchymal stem cells that are capable of differentiating into osteoblasts. Although the application of distraction forces during the distraction osteogenesis leads to subperiosteal bone, tension on the periosteum alone is sufficient to produce significant amounts of subperiosteal bone. In addition, it is possible to produce new bone formation by periosteal distraction osteogenesis (PDO) without corticotomy. According to the literature, obtaining newly formed bone is possible by mesenchymal stem cells that are under tension and capable of differentiating into osteoblasts without corticotomy and osteotomy, as in distraction osteogenesis. In the recent literature, few studies exist about periosteal distraction osteogenesis that uses nearly the same classic distraction osteogenesis protocol. It is not yet an accepted technique in humans, and to the best of our knowledge no study has evaluated the effects of the latency period on newly formed bone obtained by periosteal distraction osteogenesis.

The purpose of the present study was to develop a rabbit model for osteogenesis by periosteal distraction and to histomorphometrically define the effects of latency period on the bone formation.

MATERIALS AND METHODS
Animal care
The study protocol was reviewed and approved by the Research Review Committee and the Animal Care Committee of Gülhane Military Medical Academy (Ankara, Turkey). Thirty-six New Zealand White adult rabbits with a mean weight of 4.150 ± 0.550 kg were used as the animal model.

Distractors
The periosteal distractors were custom designed (as in the studies conducted by Schmidt et al. and Scenimen et al.), built from stainless steel and weighing 13 g each. The device consisted of a U-shaped body with 2 legs that could be rigidly fixed to the lateral aspect of the mandible. It was fixed to the mandible with 2 3-mm titanium screws. The device had a central distraction screw which is attracted to a titanium mesh plate with curved edges (Fig. 1).

Anesthesia and surgical method
Rabbits were sedated before surgery with ketamine 30 mg/kg and xylazine 3 mg/kg. Anesthesia was included and maintained by administration of 1%-3% sevoflurane mixed with oxygen via a facemask. Rabbits were in supine position, and the heart rate, respiratory rate, pulse oximetry, and temperature of the animals were monitored. Before the operation, the area was shaved and prepared with betadine, and a subcutaneous injection of 0.5% lidocaine with 1:200,000 epinephrine was given in the lateral ramus and submandibular regions.

The right side of the mandible was exposed through a submandibular incision. A periosteal flap was raised on the lateral aspect of the ramus. Three 5-mm linear incisions were made through the skin, muscle, and periosteum of the mandible with a no. 11 scalpel blade. The 2 legs and the center screw of the device were then placed through the tissue. The legs were rigidly fixed with 2 3-mm screws (Fig. 2). The periosteal flap was repositioned and the wound closed in layers using 4.0
Vicryl. Cloramphenicol ointment was applied to the wound.

Postoperative care
During the recovery period, the animals were observed until they were alert and drinking. Then 0.2 mg buprenorphine was administered intramuscularly to augment analgesia. Cefazolin (10 mg/kg) was given for 3 days.

Periosteal distraction and killing protocol
The rabbits were divided into 2 groups of 18 each according to the duration of latency period. In group 1 (control group), the latency period was 7 days, and in group 2 (experiment group) it was 1 day. In both of the groups, the distraction period started after the latency period and 7.0 mm (5 mm periosteal distraction and 2 mm clench of the distractor) periosteal distraction was achieved by turning the screw 0.25 mm twice a day for 10 days.

Both of the groups were divided into 3 subgroups according to the killing day. The rabbits were killed on the 15th, 30th and 60th days of the consolidation period (subgroups A, B, and C, respectively; Table I). Killing was done by intracardiac injection of pentobarbital after sedation (intramuscular ketamin (30 mg/kg) and xylazine (3 mg/kg) combination). After the animals were killed, their mandibles, with the soft tissues removed, were fixed in 10% neutral-buffered formalin for 1 week. After decalcification (by 10% formic acid), the distraction region was sectioned with a scalpel to create a perpendicular piece of mandible with a length of 10 mm and width of 4 mm. These pieces were stained with hematoxylin and eosin. Histologic analysis was made by a light microscope and histomorphometric measurements were performed by a computer-assisted image analysis system.

Assessment of tissues after periosteal distraction
Differences between group 1 and group 2 on the 15th, 30th, and 60th days were evaluated with the Mann-Whitney U test by paired observations. Three subgroups were separately subjected to statistical analysis by using the Mann-Whitney test followed by Bonferroni correction. P values of <.05 were accepted as statistically significant.

Histomorphometric analysis
Histologic and histomorphometric analyses were performed under a light microscope by using a digital camera. All digital images were arranged to panoramic mosaic form. Histomorphometric measurements were performed by the digital program ImageJ 1.42 (Java 1.5.0_03 [32-bit]; Fig. 3).

RESULTS
Clinical evaluation
The distractors were very stable, allowing easy distraction. After a few hours, the rabbits were able to eat independently. In all of the animals, the soft tissues were intact and normal in appearance.

Histologic analysis of periosteal distraction sites
Group 1-A. Histologic evaluation showed new bone formation on the lateral side of the mandible in the periphery of the distraction region. At the edge of the distraction site, there was evidence of new bone formation. Interstitial fat tissue was encountered between the vestibular layer of the mandible and newly formed bone under the periosteum (Fig. 4, a).

Group 1-B. Thicker newly formed bone was observed around the distractor legs in this group. Although distraction led to an increase in the number of osteocytes in the distraction site, interstitial fat tissue was still seen between the vestibular layer of the mandible and newly formed bone under the periosteum (Fig. 4, b).

Group 1-C. The periosteal proliferation of the newly formed bone tissue increased in this group. More mature lamellar bone was observed near the periosteum. Fatty tissue was seen also in this group, but it was thinner. Regular bone sequence was detected in this fatty tissue (Fig. 4, c).

Group 2-A. Newly formed bone tissue was present under the distractor’s mesh. Interstitial fat tissue was seen in the distraction area (Fig. 5, a).

Group 2-B. More newly formed bone was observed around the distractor legs in this group. Although the

<table>
<thead>
<tr>
<th>Table I. Distraction protocol</th>
<th>Group 1-A</th>
<th>Group 1-B</th>
<th>Group 1-C</th>
<th>Group 2-A</th>
<th>Group 2-B</th>
<th>Group 2-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of rabbits</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
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<td>6</td>
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<tr>
<td>Latency period, d</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Distraction period, d</td>
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<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Distraction rate, mm/d</td>
<td>0.25 × 2</td>
<td>0.25 × 2</td>
<td>0.25 × 2</td>
<td>0.25 × 2</td>
<td>0.25 × 2</td>
<td>0.25 × 2</td>
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<tr>
<td>Consolidation period, d</td>
<td>15</td>
<td>30</td>
<td>60</td>
<td>15</td>
<td>30</td>
<td>60</td>
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</table>
newly formed bone was similar to that in group 1-B, the trabeculae were thinner (Fig. 5, b).

Group 2-C. Newly formed bone was observed that was almost the same as that of cortical layer of the mandible. Thin trabeculae were observed in the periosteal distraction region (Fig. 5, c).

Histomorphometric measurements revealed that whereas the mean area of newly formed bone formation in group 1 (latency period 7 days, control group) was 2.62 cm², it was 3.26 cm² in group 2 (latency period 1 day, experiment group). However, the differences between all 1-day latency period animals and 7-day latency period awaited groups were not statistically significant (P > .05; Table II). Similarly, no significant differences between subgroups within each group were detected (P > .05; Table III). Histomorphometric analysis showed that the quantity of newly formed bone in group 2 (1-day latency period) specimens was greater than in group 1 (7-day latency period) specimens in both groups (Fig. 6).

DISCUSSION
Reconstruction of alveolar bone loss is difficult, because the deformity involves deficiencies in both the bone and mucosa. Many techniques have been used for the treatment of this deficiency. Alveolar distraction osteogenesis may be a viable alternative for the management of these situations. However, alveolar distraction osteogenesis requires a corticotomy that is difficult to perform in thin alveolar bone. In addition, although alveolar distraction osteogenesis has been shown to increase alveolar bone height,1,2 the native atrophic alveolar ridge is often knife-edged.41 Distraction osteogenesis, which has a long treatment period (latency and consolidation), may lead to some complications, such as infection, late bowing, nonunion, and refracture.49 Additionally, osteotomy or corticotomy is used to achieve new bone formation in distraction osteogenesis based on bone healing. For these reasons, we planned the present study for the evaluation of osteogenesis, without corticotomy, applied by controlled distraction of the periosteum with 1- and 7-day latency periods.

Recent animal experimental studies41-48 revealed that tensile strain acting on the periosteum is effective to produce new bone formation without corticotomy. There is no need for osteotomy or corticotomy in periosteal distraction osteogenesis. Kostopoulos and Karring42 reported that applying a Teflon capsule to the lateral surface of...
Fig. 4. Periosteal proliferation in group 1 and newly formed bone (blue arrow), fatty tissue (white arrow), and cortex of mandible (black arrow) after 15 (a), 30 (b), and 60 (c) days of consolidation. Hematoxylin and eosin, ×100.

Fig. 5. Periosteal proliferation in group 2 and newly formed bone (black arrow), fatty tissue (white arrow), and cortex of mandible (blue arrow) after 15 (a), 30 (b), and 60 (c) days of consolidation. Hematoxylin and eosin, ×100.

Table II. Comparison between groups and amount of new bone formation (cm²)

<table>
<thead>
<tr>
<th>Consolidation period, d</th>
<th>1-d latency period group</th>
<th>7-d latency period group</th>
<th>P value*</th>
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<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Median</td>
<td>Range</td>
</tr>
<tr>
<td>15</td>
<td>2.28</td>
<td>2.11</td>
<td>1.02-3.59</td>
</tr>
<tr>
<td>30</td>
<td>3.70</td>
<td>3.93</td>
<td>2.11-5.13</td>
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<tr>
<td>60</td>
<td>3.81</td>
<td>3.46</td>
<td>1.45-6.29</td>
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<tr>
<td>Overall</td>
<td>3.26</td>
<td>3.17</td>
<td>1.02-6.29</td>
</tr>
<tr>
<td>P value†</td>
<td>.098</td>
<td></td>
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</tbody>
</table>

*No significant differences between all 1-day latency period and 7-day latency period groups (P > .05).
†No significant differences between subgroups within each group (P > .05).

Table III. Comparison between subgroups within each group

<table>
<thead>
<tr>
<th>Consolidation days</th>
<th>P value*</th>
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<tr>
<td>15-30</td>
<td>.041</td>
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<tr>
<td>15-60</td>
<td>.132</td>
</tr>
<tr>
<td>30-60</td>
<td>.937</td>
</tr>
</tbody>
</table>

*According to Mann-Whitney test followed by Bonferroni correction, there were no significant differences between 15-30, 15-60, and 30-60 days’ consolidation subgroups of the main groups (P > .05).
of mandible of rats was a successful way to create new bone tissue. Kessler et al.\textsuperscript{44} tested dynamic periosteal elevation by a titanium mesh that had been implanted beneath the periosteum. They then raised it with rows of micropillars for distraction of periosteum and showed that a quantity of new bone of a similar amount was formed underneath the titanium mesh. They also claimed that the amount of bone formed by dynamic elevation seemed to be unlimited, depending on the height of the mesh. Yamauchi et al.\textsuperscript{47} examined periosteal expansion osteogenesis in an experimental study based on the concept of distraction osteogenesis by using a beta-tricalcium phosphate (β-TCP) block instead of an original bone segment. The β-TCP was distracted 0.5 mm/d for 8 days after a latency period of 8 days. They reported that newly formed bone was obtained in the gap between the lingual surface of the mandible and the β-TCP block. In another experimental study with dogs, Yamauchi et al.\textsuperscript{50} assessed the clinical outcome of periosteal expansion osteogenesis for treatment of a deficient alveolar ridge, maintenance of dental implants inserted in the distracted region, and osteocompatibility of β-TCP block regions. They reported the periosteal expansion osteogenesis with a β-TCP block to be a useful method for implant placement on deficient alveolar bone treated by this technique. Estrada et al.\textsuperscript{45} also evaluated the potential of bone formation by distraction of periosteum at 2 different rates (0.25 and 0.5 mm/d) in the first part of their study and reported that more new bone was obtained by slow rate of distraction.

Schmidt et al.\textsuperscript{41} reported that an average 2.86 mm of new bone height was formed by periosteal distraction in rabbit model. Sencimen et al.\textsuperscript{43} claimed that the mean extent of new bone formation was 14.4 mm\textsuperscript{2}. These 2 results were in line with the finding of the present study. In our study, 2.62 cm\textsuperscript{2} and 3.26 cm\textsuperscript{2} newly formed bone tissue were gained in control and experimental groups, respectively.

Oda et al.\textsuperscript{46} investigated the tissue reactions in a rabbit model by using periosteal distraction with decorcitating holes. They concluded that periosteal distraction with decorcication might be effective in promoting bone formation. In a very recent study, Sato et al.\textsuperscript{48} achieved osteogenesis by gradually expanding the interface between bone surface and periosteum in rabbit models. In that study, mesenchymal stem cells were administered into the gap, and the researchers claimed that in the experimental group the volume, height, bone mineral density, and bone mineral content increased significantly in newly formed bone tissue.

In the present study, 2 latency periods with different consolidation periods of periosteal distraction were compared. A subperiosteal region created by periosteal expansion between the titanium mesh plate and the lateral aspect of the mandible exhibited new bone formation. Histomorphometrically, newly formed bone observed in the experiment group with 1 day latency period was more than in the control group, but it was not statistically significant.

Histomorphometric analysis revealed that the quantity of newly formed bone in 30-day specimens was greater than in 15-day and 60-day specimens in the 7-day latency period group, and this was in accordance with the results reported by Sencimen et al.\textsuperscript{43} On the other hand, the quantity of newly formed bone in 60-day specimens was greater than in 15-day and 30-day specimens in the 1-day latency period group. However, this slight increase was not statistically significant.

In the present study, no differences were observed between the newly formed bone and the native bone from the 15th to 60th days of the control and experimental groups. There was also ossification in both groups, but the distraction area was rich in interstitial fatty tissues. Whereas periosteal distraction constitutes new bone formation by way of the cellular osteogenic layer of the periosteum, lack of bone marrow cells might play a role in the occurrence of fatty tissue. In our opinion, the reason for the formation of this interstitial fatty tissue may be the incision of the periosteum from 3 sites during the application of the distractor. This situation probably negatively affected the layer of periosteum. Vertical incisions that would have damaged the osteogenic activity of the periosteum may have been the reason for imposition of fatty tissue. This is in concordance with the findings of Sencimen et al.\textsuperscript{43} The distractor design, resembling Sato et al.’s\textsuperscript{48} appliance, may have less harmful effect on the periosteum.

On the other hand, according to Ilizarov,\textsuperscript{51,52} continuous or periodic stimulation must be applied to mature newly formed bone in a particular direction. But in the present study, because of the distractor design, which did not allow applying stimulation, it was not possible to apply direct stimulation for the maturation of newly formed bone. Although chewing forces could have provided the stimulation requirement for the maturation of newly formed bone, the rabbit might not use the distracted side of their jaw because of postoperative pain. Disuse of the distracted side might have negatively affected the maturity of the newly formed bone.\textsuperscript{43}

In conclusion, periosteal distraction osteogenesis is a viable method in acquired and congenital alveolar ridge defects, resulting in new bone formation. Newly formed bone was obtained by periosteal distraction osteogenesis applying different latency periods. Although periosteal distraction was applied to the lateral aspect of the mandible, it may also be applicable to an atrophic alveolar process. In further studies, periosteal distraction osteogenesis may be applied in the oral cavity.
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REFERENCES


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