Bone decortication rate and guided bone regeneration under an occlusive titanium dome: Micro-CT analysis

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Abstract

Aim: We aimed to evaluate how the rate of bone decortication influences guided bone regeneration.

Material and Methods: Twenty-four titanium domes were placed bilaterally on the parietal bones of sixteen New Zealand rabbits after drilling the bone with a small round burr to induce bleeding from the marrow space (Group A: one hole; Group B: three holes; Group C: nine holes), while the bone surface was left intact at the control sites (Control group). Each group included six samples. Bone decortication rates were approximately 4% in group A, 12% in group B, and 36% in group C. All rabbits were killed after a 12-week bone-healing period, and results were evaluated by micro-CT.

Results: Both newly generated tissue formation and mineralized bone formation were higher in all experimental groups than in the control group (P < 0.05), increasing in proportion to the increasing number of holes to a peak in Group C.

Conclusion: Bone decortication improves mineralized bone and newly regenerated augmented tissue during guided bone regeneration. Bone decortication can be used as an integral part of guided bone regeneration procedures. Also more bone decortication rate seems to be more effective.

Keywords: Guided Bone Regeneration; Titanium Dome; Bone Decortication.

INTRODUCTION

Various degrees of bone insufficiencies can occur in the maxillofacial region as a result of infection, trauma, tooth extraction, periodontal pathology, several oral and maxillofacial surgery procedures, congenital deformities, and physiological bone resorption in the toothless alveolar bone regions (1-3). Different graft materials and surgical protocols have been used to restore bone volume, each with various advantages and disadvantages. One method is guided bone regeneration (GBR) (2). GBR is known as 'guided bone augmentation' (GBA) when new bone is induced on the adjoining bone surface and beyond the original outer skeletal envelope (4). The efficacy of supplementing bone production in GBA procedures by GBR has been explored in many experimental animals (5-11) and in humans (6,12,13).

GBR is based on maintenance of the cavity during bone formation by preventing undesired epithelial and connective tissue migration in a defined area adjacent to the bone surface, while allowing select pluripotent and osteogenic cells to enter the GBR-treated site. To protect against ingrowth of fibrous connective tissue, different kinds of barrier membranes have been used (1,4,7,8,12,14). Adequate primary wound closure, space creation and maintenance with immobilized biocompatible barrier material of sufficient rigidity, elimination of epithelium and connective tissue, a healthy vascularized bone bed, stability of the initial blood clot, and an appropriate healing time all influence the success of GBR (1,2,8).

As in other body tissues, a vascular blood supply is needed for new bone formation. Hematopoietic cells, osteoprogenitor cells, and various growth factors and cytokines mediate the new bone formation and are transported to the target area through the blood. Thus, vascularization or angiogenesis constitutes the first phase of ossification. Cortical bone decortication or perforation improves bleeding and clot formation, allowing the migration of osteogenic progenitor cells; this may provide a therapeutic advantage (1,7).

Bone decortication prior to insertion of a bone graft is
frequently performed as part of a GBR procedure; however, the effect of decortication on new bone formation in GBR remains unclear (1,15). While some authors advocate surgical decortication of the bone (9,15,17), other authors (18-21) suggest bone regeneration can occur without decortication. The question remains: is decortication of bone necessary or optional?

Bone decortication is generally performed by drilling holes through the cortical bone into the cancellous bone or by removing the cortical bone completely (1). The literature provides no guidance on the number of holes that should be made in the cortical bone or the size of the area that should be decorticated relative to the size of the GBR-treated site. In this study, our purpose was to characterize the effect of cortical bone perforation rate in an experimental model of GBR.

MATERIAL and METHODS

Twelve adult male New Zealand white rabbits (7–8 months old; average weight 3.5 kg) were used in this study. Ethical approval of experimental design was obtained by the Animal Ethics Committee of Inonu University (2014/A-44).

Surgery was performed under general anesthesia administered with intramuscular injections of 10 mg/kg xylazine hydrochloride (Xylazinbio 2%, Boveta PLC, Ivanovice na Hane, Czech Republic) and 50 mg/kg ketamine hydrochloride (Alfamine 10%, Alfasan International, Woerden, Holland). Under sterile conditions, a 4–5 cm midsagittal incision was made from the nasal bone to the occipital region through the skin and periosteum to raise a cutaneo-periosteal flap from the forehead. Then, the flaps were reflected laterally with a periosteal elevator and the parietal bones were seen bilaterally on both sides of the midline. Custom-made, smooth surface, standardized stiff domes of pure titanium (inner diameter 8 mm, inner height 4 mm, 0.3 mm thick) were used a barrier.

Two titanium domes were placed bilaterally on the parietal bones of each rabbit. Prior to dome placement, the cortical bone was drilled under copious sterile physiological solution (0.9% NaCl) irrigation using a small round burr (diameter, 1.6 mm) in a standard manner to induce bleeding from the marrow space at the experimental sites (Group A: one hole; Group B: three holes; Group C: nine holes), while the bone surface within the domes was left intact at the control sites (Control group) (Figure 1). Decortication rates corresponded about 4% in group A, 12% in group B, and 36% in group C. Including two different groups in each animal, assignment of the sites to receive the experimental or control treatment was done on a random basis. The borders of the titanium domes were glued to the skull with N-butyl-2-cyanoacrylate (Histoacryl; B. Braun, Melsungen, Germany) to ensure peripheral sealing between the dome margins and the cortical bone surface (Figure 2). Then, the domes were covered by carefully replacing the periosteum and scalp as much as possible, and the flap was sutured hermetically. For wound cleaning, all animals were examined every other day for 10 days. All rabbits were sacrificed with an overdose of 200 mg/kg IV pentobarbital sodium (Pentothal Sodium; Abbott, North Chicago, IL) after a 12-week bone-healing period.

Figure 1. Decortication ratios of the groups. (Control group: No decortication, Group A: One hole decortication, Group B: Three hole decortication, Group C: Nine hole decortication)

Figure 2. Bonding the dome to parietal bones

Micro-CT analysis

Specimens were taken from the experimental areas of the cranium for micro-CT evaluations (Figure 3). Test specimens were scanned with micro-Ct system (Skyscan 1172; Bruker-microCT, Kontich, Belgium). System was set at 100 kV and 100 µA using a 0.5-mm Al+Cu filter with an image resolution of 13.68 µm pixels. Total of 60 min scanning was completed for each specimen with 180° rotation around the vertical axis, camera exposure time of 1900 ms, rotation step of 0.4°, frame averaging of 2, and a random movement of 15.

The resulting two-dimensional images (8-bit TIFF) were used to reconstruct axial cross-sections using NRecon v.1.6.3 (Brucker-microCT) with a beam hardening correction of 50%, smoothing of 3, and an attenuation correction applied.
coefficient range of $0.0–0.0550$. CTAn v.1.12 software (Bruker-microCT) was used for volumetric analysis. Three-dimensional visualization and qualitative evaluation were performed with CTVol software (Bruker-microCT). The following parameters were calculated for each specimen: (1) the volume of newly generated all augmented tissue (NTv); (2) the total experimental volume (TAv) (volume of the hemispherical titanium dome = $\frac{4}{3}\pi r^{3}/2 = \frac{4}{3} \times 3.14 \times (4 \text{ mm})^{3}/2 = 133.97 \text{ mm}^{3}$); (3) the volume of the amount of mineralized bone in the NTv (MBv); (4) the percentage of newly generated augmented tissue volume (NTv%): $\text{NTv}/133.97 \times 100$, and (5) percentage of mineralized bone volume (MBv%): $\text{MBv}/\text{NTv} \times 100$.

**Figure 3.** Titanium domes were taken from calvarial bone with parietal bones after rabbits were sacrificed.

**Statistics**

All data were analyzed using a commercially available software package program (SPSS 21.0, SPSS Inc., Chicago, IL). Kruskal–Wallis test was used for intergroup comparisons. When there were differences between groups, data were analyzed by Mann–Whitney U-test. Differences of $P < 0.05$ were considered significant.

**RESULTS**

**Clinical observations**

The health status of all rabbits remained good throughout the 12-week healing period after surgery. All experimental sites demonstrated uneventful healing with no signs of infection or adverse reactions upon dissection and removal of the cutaneous layers above the domes. All titanium domes were immobilized in their original position. Macroscopic examination revealed clear volumetric differences between the control and experimental groups (Figure 4).

**Micro-CT**

The results of micro-CT analyses are shown in Tables 1, note that the results correlated well (Figure 5). The volumetric measurements of both newly generated tissue formation and mineralized bone formation were higher in all experimental groups than in the control group ($P < 0.05$). These values increased with the increasing number of holes in the experimental sites and reached the peak in Group C. However, the volumetric parameters did not differ between groups B and C ($P > 0.05$).

**Table 1. Micro-CT analysis of newly generated augmented tissue (NT) and newly mineralized bone formation (MB). $^*p<0.05$**

<table>
<thead>
<tr>
<th></th>
<th>MB Mean ± SD</th>
<th>MB% Mean ± SD</th>
<th>NT Mean ± SD</th>
<th>NT% Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Group</td>
<td>4.31 ± 1.73</td>
<td>10.18 ± 1.53</td>
<td>44.52 ± 24.26</td>
<td>33.22 ± 18.10</td>
</tr>
<tr>
<td>Group A</td>
<td>15.38 ± 7.15</td>
<td>18.31 ± 5.91</td>
<td>82.25 ± 16.43</td>
<td>61.38 ± 12.25</td>
</tr>
<tr>
<td>Group B</td>
<td>24.27 ± 7.67</td>
<td>26.25 ± 3.96</td>
<td>90.93 ± 20.13</td>
<td>67.86 ± 15.03</td>
</tr>
<tr>
<td>Group C</td>
<td>28.59 ± 6.21</td>
<td>28.59 ± 3.88</td>
<td>100.15 ± 17.02</td>
<td>74.74 ± 12.70</td>
</tr>
<tr>
<td>Kruskal–Wallis test</td>
<td>0.003*</td>
<td>0.002*</td>
<td>0.029*</td>
<td>0.029*</td>
</tr>
<tr>
<td>Mann–Whitney U-test</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control-A</td>
<td>0.010*</td>
<td>0.010*</td>
<td>0.038*</td>
<td>0.038*</td>
</tr>
<tr>
<td>Control-B</td>
<td>0.010*</td>
<td>0.010*</td>
<td>0.038*</td>
<td>0.038*</td>
</tr>
<tr>
<td>Control-C</td>
<td>0.010*</td>
<td>0.010*</td>
<td>0.019*</td>
<td>0.019*</td>
</tr>
<tr>
<td>A-B</td>
<td>0.065</td>
<td>0.026*</td>
<td>0.394</td>
<td>0.394</td>
</tr>
<tr>
<td>A-C</td>
<td>0.015*</td>
<td>0.015*</td>
<td>0.078</td>
<td>0.078</td>
</tr>
<tr>
<td>B-C</td>
<td>0.485</td>
<td>0.310</td>
<td>0.699</td>
<td>0.699</td>
</tr>
</tbody>
</table>
The literature provides inadequate and inconsistent cases without decortication (28-30). The effect of decortication on incorporation of onlay bone-material and bone. Many studies have demonstrated the may increase the physical adherence between graft blood vessels and the GBR site (1). Moreover, decortication site (29). This procedure may provide a direct link between induced by decortication and can migrate into the surgical (28). Growth factors, cytokines, and other precursors are promoted bleeding, and accelerates bone integration (2). Consistent with prior studies (9,14,20,22,23), this study demonstrated that it is possible to augment the bone generation into areas where previously there was no bone present by using a titanium cap. Furthermore, new bone formation and mineralized bone tissue gradually improved with increasing numbers of penetrations of the rabbit skull after a healing period of three months.

Alveolar bone loss is one of the most challenging aspects of oral and maxillofacial surgery. To promote bone volume, many techniques have been used in the clinical setting. If a patient has insufficient bone height or width, surgical GBR procedures may be used to augment bone (24). The GBR technique includes separation of the epithelium and connective tissue, maintenance of the space, stability of the blood clot, and primary wound closure (25). GBR procedures are successful in the presence or absence of bone graft materials.

Regeneration of new bone depends on the development of new blood vessels that provide stimulation and nutrition to the surgical site. Schmid et al (26), demonstrated a correlation between new bone formation and angiogenesis. Vascular endothelial growth factor (VEGF) and osteocalcin (OC) are critical factors to neovascularization and osteogenesis. Lee et al (27), demonstrated greater VEGF and OC expression in the study group than controls at 2 and 4 weeks after surgery, although the differences were not significant. Thus, we suggest the GBR space should have access to the vascular tissues.

Decortications of the bone exposes the bone marrow, promotes bleeding, and accelerates bone integration (28). Growth factors, cytokines, and other precursors are induced by decortication and can migrate into the surgical site (29). This procedure may provide a direct link between blood vessels and the GBR site (1). Moreover, decortication may increase the physical adherence between graft material and bone. Many studies have demonstrated the effect of decortication on incorporation of onlay bone-grafting and bone healing, noting better results than in cases without decortication (28-30).

The literature provides inadequate and inconsistent information in experimental animal models in terms of the impact of decortications on GBR. It is difficult to assess the available data because of differences in surgical and animal models, and in the size, architecture, and contents of GBR appliances, assessment intervals, and aspects of GBR (1).

Many experimental animal studies have explored different aspects of GBR techniques in which bone decortication was not used as a part of the surgical protocol (1). Lundgren et al (20), evaluated the augmentation of intramembranous bone beyond the skeletal envelope by placing a titanium dome with an inner diameter of 4.5 mm and an inner height of 3.0 mm on the skull of rabbits. They used a circular slit and peripheral horizontal flange to achieve a tight fit without perforations. After a three-month healing period, they found complete bone fill in all domes, regardless of decortication of the calvarial bone. In our study, complete bone fill of created space did not occur with three months in any of the experimental or control groups. This apparent discrepancy is likely attributable to the difference in dome size. Kostopoulos et al (19), used a rat model to assess the possibility of developing bone tuberosities on the mandibular ramus by using a rigid polytetrafluoroethylene occlusive capsule 4.5 mm high and 9 mm wide. Neither decortications nor circular grooves were made in the experimental model. The mean bone fill reached 41.6% and 52.2% in the two-month and four-month specimens, respectively. Other studies (31,32) used nearly identical experimental model and showed amounts of new bone in the capsules reached almost 100%. In contrast, the mean amount of bone attained in our control group was about 33% of the total space created by the domes after three months. Differences between these prior studies and our results may be attributable to differences in the assessment interval, distinct surgical bone models, and animal species. When compared to rabbits, rats are members of a lower phylogenetic species with a characteristically higher capacity to regenerate new bone (1,21). Our study and the abovementioned animal studies sought to achieve bone formation beyond the normal anatomic limits of the skeleton by means of various GBR procedures and showed that the amount of new bone tissue increases to different degrees without decortication and without extra applications to enhance bone healing potential.

Although the effect of decortication on bone formation beyond the skeletal envelope remains controversial, a group of researchers (15,33,34) found a significant improvement in bone regeneration after decortication. Min et al (33), examined new bone formation with only decortications of the rabbit calvarium using a titanium cap GBA model. At the experimental site, cortical decortications were performed mechanically with a number 4 round bur, whereas the bone surface on the control site was left intact. The penetration rate was 28% for the experimental site. At three months, the percentage of newly generated tissue and mineralized bone was significantly greater in the decortication group (78.9% vs. 69.8% and 24% vs.
16.4%, respectively). In another study, Majzoub et al (34), positioned titanium domes on the cortical surface of rabbit calvaria and mechanically perforated the skull with a carbide bur to determine whether decortications of the calvarial bone would enhance bone augmentation. At all evaluation intervals, increased bone fill was noted within the experimental and control domes. Perforated and non-perforated sites yielded significant differences in bone fill after two months (71.72% vs. 53.58%). In a rat GBA model, Rompen et al (15), placed 5-mm long, 4-mm wide, 3-mm high titanium chambers in the rat parietal bone. In the decortication group, the calvarium was penetrated with nine 0.8-mm diameter holes (penetration rate 22.6%). After four months, bone augmentation at decorticated sites was significantly higher in comparison to sites that were left intact (172.8% vs. 141%, this refers to newly generated bone height relative to calvarial thickness). In this study, we observed that both mineralized and newly formed bone tissue were significantly higher in all decortication groups. However, the studies performed by Lundgren et al (7) and Slotte and Lundgren (21) placed titanium cylinders on the skull of rabbits and found no significant difference in bone augmentation in decorticated versus control sites after a healing period of three months. This discrepancy may be due to the placement of the two mini-screws inserted for cylinder immobilization and peripheral sealing. Stress created by the mechanical forces transmitted through the adjoining bone tissue via titanium lid may stimulate bone regeneration. Frost (35) suggested that a noxious stimulus increased bone turnover and lead to faster bone regeneration.

Nishimura et al (17), examined the impact of decortication size on bone regeneration in a rabbit GBR model using titanium mesh-reinforced expanded polytetrafluoroethylene barriers. They decorticated the skull in two different sizes (1 × 15 mm and 3 × 15 mm slits). Rabbits were sacrificed at different time intervals. At the end of the study, they found that the larger slits were initially associated with a shorter filling time and higher new bone formation, but there were no differences in the amount of newly formed bone 12 weeks after therapy. In another study, Min et al (36), used microCT to assess the effect of penetration rate on bone healing within a titanium cap (the same size and shape used in this study) in one rabbit calvarium. At the experimental and control sites, the skull bone was penetrated with nine smaller holes using a no. 4 round bur (penetration rate 28%) and a no. 2 round bur (penetration rate 14%). Titanium caps were fixed in circular grooves. After 11 weeks, they found complete bone fill in both titanium caps and concluded that quantity of amount and mineralization of newly formed bone did not vary with penetration rate. Consistent with this work, we found that penetration rates of 12% and 36% yielded insignificant differences in bone amount and mineralization, although we did not achieve complete dome fill after 12 weeks.

To obtain bone regeneration/formation, titanium barriers/meshes/caps are typically used (5,37,38). Stabilization of the GBR site is crucial for ideal healing (1). Nishimura et al (17), stabilized an expanded polytetrafluoroethylene (e-PTFE) membrane reinforced with a thin titanium mesh with titanium pins. In many studies (7,8,20,23), circular grooves have been used to fit and peripherally seal the titanium barriers on the adjoining bone. Newly generated bone under the empty titanium cap appears to originate from the circular grooves (33). In this study, we used N-butyl-2-cyanoacrylate d to fix the titanium caps to the skull. As a tissue adhesive, N-butyl-2-cyanoacrylate provides immediate hemostasis, a bacteriostatic effect, and biocompatibility (39). This method prevented the application of extra forces that promote bone healing. In this manner, we attempted to explore only the effect of decortication rate on bone gain in GBR.

CONCLUSION

This study showed that GBR using a titanium barrier in a rabbit calvarial model with and without decortication may result in bone formation. Decortication improved the amount of newly augmented bone tissue and mineralized bone formation after three months. Thus, decortication was effective in promoting bone formation with GBR. Volumetric analyses demonstrated increasing new bone formation with increasing decortication rate, and a limited further increase was observed from 12% decortication (three holes) to 36% (nine holes). We used standard holes in experimental groups but need to explore the effects associated with varying hole sizes, as the size of the hole may be as important as the number for angiogenesis and osteogenesis. Additional controlled animal and human studies are needed to determine the size and shape of bone marrow penetration holes, and the optimal decortication area relative to the size of the GBR-treated site for ideal decortication. Within the limitations of this study, we conclude that decortication of the bone during GBR improves mineralized bone formation and newly regenerated augmented tissue formation, even if only one hole is drilled. In other words, decortication, which has no negative effect on surgery, can be used as an integral part of GBR procedures.

Competing interests: The authors declare that they have no competing interest.

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