Resorption of alveolar bone caused by periodontal disease or trauma results in severe horizontal and vertical bone defects. Bone reconstruction at the edentulous ridge is essential when considering implant-supported removable or fixed restorations. In order to restore severe bone defects including vertical bony deficiency, many different augmentation methods have been introduced.1–7

A solid space maker in the ridge requiring vertical augmentation is essential to achieve bone regeneration. Autologous block bone graft is considered to be the gold standard for vertical bone augmentation due to its osteoinductive and osteoconductive ability.8 However, there are many known drawbacks associated with harvesting autogenous bone. It requires additional surgery with delayed surgery and increased surgical costs, fracture of the donor site, and neurosensory disturbances at the donor site.8–12

Allogeneic block bone was introduced for vertical ridge augmentation as an alternative to autogenous bone.13–15 However, allogeneic block bone is also known to have some drawbacks, such as fast bone resorption, risk of cross contamination from human origin, and high cost.16–18

As an alternative to autogenous or allogeneic bone graft, the autologous demineralized/undemineralized tooth bone graft has recently been introduced. Dentin is known to have similar inorganic and organic components to alveolar bone.19 Tooth bone has been utilized in the form of particulate or block bone with and without demineralization for ridge augmentation.20

Comparative Histomorphometric Evaluation of Bone Regeneration with Different Preparations of Xenogeneic Tooth Block Bone

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Purpose: The aim of this animal study was to evaluate new bone formation in human dentin block grafted on rabbit calvaria according to a comparison of histologic analysis. Materials and Methods: Human teeth were prepared according to four different types of dentin blocks: group 1, demineralized and microperforated dentin block; group 2, demineralized dentin block; group 3, undemineralized and microperforated dentin block; group 4, undemineralized dentin block. These four different dentin blocks were grafted on nine rabbit calvaria, and animals were sacrificed at 2, 4, and 8 weeks after the surgical procedure for histologic evaluation. Results: In group 1, histologically, new bone formation was initiated at the interface between demineralized and microperforated dentin block and host bone and microholes at 2 weeks, and mature bone was observed at 8 weeks. In group 3, new bone formation was observed at 8 weeks in the undemineralized and microperforated dentin block bottom and microholes. The bone formation ratio of group 1 was significantly higher at 2, 4, and 8 weeks compared with groups 2, 3, and 4 (P < .05). The bone formation ratio in microholes at 2 and 8 weeks in group 1 was significantly greater than in group 3 (P < .05). Conclusion: New bone formation of the demineralized dentin block began more rapidly than the undemineralized dentin block, and perforated dentin block was more effective in bone formation than dentin block without microholes. INT J ORAL MAXILLOFAC IMPLANTS 2019;34:1413–1422. doi: 10.11607/jomi.7290

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Demineralization of particulate dentin is known to release diverse growth factors to induce bone regeneration. Undemineralized tooth has demonstrated delayed osteoinductive properties because hydroxyapatite may block the release of diverse growth factors from dentin. However, studies about the efficacy of demineralization of tooth block bone for bone regeneration are rare.

The aim of the present research was to evaluate, according to different preparation methods of dentin block, bone reformation in dentin block bone grafted on rabbit calvaria by comparative histologic analysis.

MATERIALS AND METHODS

Preparation of Dentin Blocks for Grafts

Extracted human permanent molars were collected at the Department of Oral and Maxillofacial Surgery, Catholic University Medical Center of Daegu. Teeth with decay or restorations were excluded in this research. After removal of soft tissue, calculus, and pulp attached to teeth with a rotary bur, the enamel portion was removed with a fissure bur and teeth were dissected into two pieces with a disk. All cutting procedures were made under saline irrigation to prevent possible denaturalization of the proteins in dentin with heat. Thirty-six dentin blocks measuring approximately 6 mm wide and 2 mm thick were prepared for four different groups:

- Preparation of dentin block group 1, demineralized and microperforated dentin block: Six to seven microholes (0.5 mm wide) were made to each dentin block using a small round bur. The perforated dentin blocks were sterilized with sterilization reagent (peracetic acid ethanol solution) in a vacuum-ultrasonic device (VacuaSonic System, CosmoBioMedicare). After sterilization of perforated dentin blocks, they were demineralized for 70 minutes using 0.6-N hydrochloride in a vacuum-ultrasonic device. The dentin blocks underwent a washing process with phosphate-buffered saline for 10 minutes and then were washed again with distilled water for a further 10 minutes in a vacuum-ultrasonic device.
- Preparation of dentin block group 2, demineralized dentin block: Dentin blocks without microperforations underwent the same processing method including sterilization, demineralization, and washing as group 1.
- Preparation dentin block group 3, undemineralized and microperforated dentin block: Dentin blocks were prepared with microholes 0.5 mm wide using a small round bur, then sterilized with peracetic acid ethanol solution and washed with phosphate-buffered saline and distilled water in a vacuum-ultrasonic device.
- Preparation of dentin block group 4, undemineralized dentin block: No microperforations were made through the dentin block for this group. The dentin blocks were sterilized with peracetic acid ethanol solution and washed with phosphate-buffered saline and distilled water in a vacuum-ultrasonic device.

Surgical Procedures

Nine adult male New Zealand white rabbits that weighed between 2.8 and 3.2 kg (average 3.0 kg) were included in the animal study. This study was approved by the Animal Care and Use Committee at the Catholic University Medical Center of Daegu. The nine rabbits were randomly divided into three groups, so three rabbits were included in each group. All rabbits received the same surgical procedures. Intramuscular injection of Ketamin (30 mg/kg, Ketalar, Yuhan) and Xylazine (10 mg/kg, Rompun, Bayer Korea) was performed to induce general anesthesia before surgery. After injecting lidocaine with 1:100,000 epinephrine along the planned skin and periosteal incisions, a sharp incision through skin and periosteum was made to expose the cranial bone. Four different types of dentin blocks were grafted on the exposed calvaria in an onlay form. On the left side, demineralized dentin blocks were placed on the left side, and undemineralized dentin blocks were grafted on the right side.
were used to close the incised tissues for closure. Intramuscular administration of Gentamycin (20 mg/kg, Donghwa) was provided for 3 days to prevent postoperative infection.

Tissue Preparation
The rabbits were sacrificed at 2, 4, and 8 weeks under general anesthesia. The calvaria was segmented with a saw and fixed with neutral buffered formalin for 24 hours. Afterward, all specimens were washed with 0.1 M phosphate buffer solution, and then decalcified with 10% formic acid for 10 days. The specimen was embedded in paraffin (Paraplast, Oxford) and sliced coronally into serial sections 5 μm thick. The specimen was stained with hematoxylin and eosin and Masson’s trichrome stains, and examined under microscopy.

Histomorphometric Analysis
Twenty fields were selected randomly from each group and photographed using the AxioCam MRc5 (Carl Zeiss) interfaced with the Axiophot Photoscope (Carl Zeiss). The AxioVision SE64 (Carl Zeiss) program was used for analysis.

The histomorphometric parameters were defined as follows:

1. Host bone to graft contact ratio (%) was determined as the ratio of the contact length, including newly formed bone, fibrous tissue, and vascular tissue relative to the total length from the bottom to the hole (groups 1 and 3) or the total length of the bottom (groups 2 and 4) of the dentin block.
2. Bone formation ratio (%) was analyzed as the ratio of the contact length of the newly formed bone to the total length from the bottom to the microhole or the total length of the bottom of the dentin block.
3. Bone formation ratio in microholes (%) was analyzed as the ratio of the contact length of the newly formed bone to the total length from the bottom to the microhole of the dentin block (groups 1 and 3).

Statistical Analysis
For the data processing and statistical evaluation, appropriate validated software was used (SPSS, version 25.0, SPSS). The statistical significance of differences between intragroup and intergroup was evaluated by one-way analysis of variance (ANOVA) with Tukey’s method. The quantitative results were expressed as means ± standard deviation, and the P value of < .05 indicated statistical significance.

RESULTS
Histologic Analysis
The tooth dentin blocks implanted in the rabbit calvaria were attached to the calvaria. The rabbit calvaria and the tooth dentin blocks were lightly stained and differentiated from surrounding tissue using hematoxylin and eosin and Masson’s trichrome stains. No signs of inflammation were indicated in any of the four groups by hematoxylin and eosin and Masson’s trichrome stains (Figs 2 to 9).

At 2 Weeks
Newly formed bone and fibrovascular tissue were observed in microholes and at the interface between demineralized and microperforated dentin block and calvaria in group 1 (Figs 2a and 2c), and many osteoblasts were observed on the surface of the newly formed bone (Fig 4a). In group 2, newly formed bone and connective tissue occupied half of the demineralized dentin block bottom (Figs 2b
and 2d). New bone formation was revealed on the calvaria-demineralized dentin block contact surface, and some osteoblasts were observed on the surface of the newly formed bone (Fig 4b). In group 3, only part of the undemineralized and microperforated dentin block bottom and microholes were attached to fibrovascular tissue (Figs 3a and 3c). There was no new bone formation in microholes, and the calvaria-undemineralized and microperforated dentin block contact surface and fibrous and vascular tissue filled the microholes (Fig 4c). In group 4, only part of the undemineralized dentin block bottom was attached to the connective tissue (Figs 3b and 3d). The calvaria-undemineralized dentin block contact surface was filled with fibrous and vascular tissue (Fig 4d).

**At 4 Weeks**

In group 1, most of the demineralized and perforated dentin block (DPDB) bottom and microholes revealed newly formed bone. New bone formation was also occurring on the surface of demineralized and microperforated dentin block (Figs 5a and 5c). More new bone formation was observed in microholes of demineralized and microperforated dentin block, and many osteoblasts were observed on the surface of the newly formed bone (Fig 7a). In group 2, bone formation was observed in half of the demineralized dentin block bottom, and fibrovascular tissue was observed in the other half (Figs 5b and 5d). Newly formed bone was revealed on the calvaria-demineralized dentin block contact surface, and many osteoblasts were observed on the surface of the newly formed bone (Fig 7b).
**Fig 5** Low-magnification images of the rabbit calvaria at 4 weeks after surgery in (a, c) group 1 and (b, d) group 2. The boxed areas are presented at a higher magnification in Fig 7. RC = rabbit calvaria; DPDB = demineralized and perforated dentin block; DDB = demineralized dentin block ([a, b] H&E stain, ×20; [c, d] Masson’s trichrome stain, ×20).

![Image of Fig 5](image1)

**Fig 6** Low-magnification images of the rabbit calvaria at 4 weeks after surgery in (a, c) group 3 and (b, d) group 4. The boxed areas are presented at a higher magnification in Fig 7. RC = rabbit calvaria; UDPDB = undemineralized and perforated dentin block; UDDB = undemineralized dentin block ([a, b] H&E stain, ×20; [c, d] Masson’s trichrome stain, ×20).

![Image of Fig 6](image2)

**Fig 7** Images showing bone formation in microholes and at the host bone to graft contact surface at 4 weeks in (a) group 1, (b) group 2, (c) group 3, and (d) group 4. (a) More new bone formation was observed in the microholes of DPDB, and many osteoblasts (arrows) were observed on the surface of the newly formed bone. (b) Newly formed bone was revealed on the calvaria-DDB contact surface, and many osteoblasts (arrows) were observed on the surface of the newly formed bone. (c) Partial new bone formation was observed, and most of the microholes and the calvaria-UDPDB contact surface were filled with fibrous and vascular tissue. (d) New bone formation and some osteoblasts (arrows) were observed in some of the calvaria-UDDB contact surfaces. N = newly formed bone; RC = rabbit calvaria; DPDB = demineralized and perforated dentin block; DDB = demineralized dentin block; UDPDB = undemineralized and perforated dentin block; UDDB = undemineralized dentin block ([a–d] H&E stain, ×100).
group 3, bone formation was visible in only a portion of the undemineralized and perforated dentin block (UDPDB) bottom, and most of the microholes and bottoms were attached to fibrovascular tissue (Figs 6a and 6c). Partial new bone formation was observed, and most of the microholes and calvaria-undemineralized and microporferated dentin block contact surfaces were filled with fibrous and vascular tissue (Fig 7c). In group 4, bone formation was observed only in part of the undemineralized dentin block bottom, and most was attached to fibrovascular tissue (Figs 6b and 6d). New bone formation and osteoblasts were observed on some of the calvaria-undemineralized dentin block contact surface (Fig 7d).

**At 8 Weeks**

In group 1, bone and fibrovascular tissue were well attached to demineralized and microporferated dentin block bottoms and microholes. In some of the bottoms of demineralized and microporferated dentin block, resorption of demineralized and microporferated dentin block was observed (Figs 8a and 8c). Newly formed bone and osteoblasts were observed at the calvaria-demineralized and microporferated dentin block contact surface, and osteoclasts were found at the demineralized and microporferated dentin block surface (Fig 10a). The hole was filled with mature bone, and osteoblasts were observed on the surface of the mature bone (Fig 10b). In group 2, the demineralized dentin block bottom was attached to the newly formed bone and fibrovascular tissue, and partial resorption of demineralized dentin block was observed (Figs 8b and 8d). Significant new bone formation and many new osteoblasts were observed on the calvaria-demineralized dentin block contact surface (Fig 10c). In most of the demineralized dentin block bottoms and microholes in group 3, newly formed bone and fibrovascular tissue were well formed (Figs 9a and 9c). In some of the microholes, a large amount of new bone formation and osteoblasts were observed (Fig 10d), while others were filled with fibrous and vascular tissue (Fig 10e). In group 4, most of the demineralized dentin
block bottoms were observed with new bone formation and fibrovascular tissue (Figs 9b and 9d). Newly formed bone was found on the calvaria-demineralized dentin block contact surface, and many osteoblasts were observed on the surface of the newly formed bone (Fig 10f).

**Histomorphometric Analysis**

In group 1, the host bone to graft contact ratio at 2, 4, and 8 weeks was 64.95 ± 4.42, 85.16 ± 3.27, and 94.73 ± 4.57, respectively. In group 2, the host bone to graft contact ratio at 2, 4, and 8 weeks was 44.46 ± 5.14, 78.90 ± 3.61, and 93.96 ± 2.90, respectively. In group 3, the host bone to graft contact ratio at 2, 4, and 8 weeks was 36.05 ± 6.26, 55.56 ± 5.73, and 88.92 ± 3.55, respectively. In group 4, the host bone to graft contact ratio at 2, 4, and 8 weeks was 24.97 ± 4.84, 30.59 ± 8.52, and 84.01 ± 4.36, respectively. One-way ANOVA and post hoc comparisons showed that the host bone to graft contact ratio at 2 and 4 weeks was significantly different in all groups, with $P < .05$. The host bone to graft contact ratio at 8 weeks in groups 1 and 2 was significantly greater than in groups 3 and 4, with $P < .05$ (Fig 11).

In group 1, the bone formation ratio at 2, 4, and 8 weeks was 50.53 ± 2.80, 54.74 ± 2.05, and 76.55 ± 3.13, respectively. In group 2, the bone formation ratio at 2, 4, and 8 weeks was 29.10 ± 2.97, 45.56 ± 3.98, and 66.44 ± 3.12, respectively. In group 3, the bone formation ratio at 2, 4, and 8 weeks was 7.12 ± 2.49, 16.01 ± 5.29, and 49.45 ± 6.68, respectively. In group 4, the bone formation ratio at 2, 4, and 8 weeks was 7.95 ± 2.56, 12.97 ± 2.87, and 41.94 ± 2.92, respectively. One-way ANOVA and post hoc comparisons showed that the bone formation ratio at 2 and 4 weeks in group 1 was significantly greater than in groups 2, 3, and 4, with $P < .05$. The bone formation ratio at 2 and 4 weeks in group 3 was not significantly different than in group 4, with $P < .05$. The bone formation ratio at 8 weeks was significantly different in all groups, with $P < .05$ (Fig 12).

In group 1, the bone formation ratio in microholes at 2, 4, and 8 weeks was 44.96 ± 5.38, 51.62 ± 2.51, and 74.05 ± 9.08, respectively. In group 3, the bone formation ratio in microholes at 2, 4, and 8 weeks was 2.32 ± 1.35, 8.12 ± 2.87, and 54.05 ± 6.12, respectively. One-way ANOVA and post hoc comparisons showed that the bone formation ratio in microholes at 2 and 4 weeks was significantly different than in group 1, with $P < .05$ (Fig 13).

**DISCUSSION**

Autologous cortical bone or cortico-cancellous block bone grafts have been widely utilized for the reconstruction of bony defects in graft-supported oral
rehabilitation due to their fast bone regeneration and slow resorption compared with bone grafts from an endochondral origin. However, grafting of intraoral autoblock bone is known to cause numerous complications. Hematoma and inflammation at the donor and recipient sites are the most common complications associated with the harvesting procedure for intraoral autoblock grafts. Other complications have also been reported, including decreased volume at the recipient site, unpredictable resorption after healing, failure of the autograft, fracture of the mandible during harvesting of autogenous block bone, and neurosensory disturbances at recipient sites.

To overcome reported disadvantages of autologous block bone, several alternative block bone grafts, including allogeneic, xenogeneic, and autologous tooth block bone grafts, have been introduced. Allogeneic block bone graft is known to have several advantages such as biocompatibility, no donor site morbidity, and unlimited volume in quantity. However, allogeneic block bone grafts demonstrated faster resorption during the healing period, cracking of the bone graft due to occlusal force, and poor integration of grafts compared with autologous block bone grafts. Bovine block has also been used as a solid scaffold for ridge augmentation. According to animal and clinical studies on block bone substitutes, bovine block revealed good osteoconductivity similar to their particulate bone substitutes, but poor biodegradation and replacement resorption were revealed. Therefore, block bone substitutes do not seem to be appropriate for vertical ridge augmentation.

Recently, as an alternative to autologous bone grafts, dentin block bone with demineralization or undemineralization has been suggested for sinus and ridge augmentation because it has osteoinductive potential after the demineralization process. Autogenous undemineralized dentin root block implanted in host bone grafts was replaced gradually by newly formed bone and showed a structural and biologic potential as an alternative to autogenous block graft. However, undemineralized dentin block demonstrates poor osteoinductive potential in nonosteogenic areas. Demineralization of the dentin bone is known to have an important effect on bone regeneration. Demineralization increases the number of exposed dentinal tubules, enlarges dentinal tubules to expose osteoinductive proteins, and decreases the crystallinity of dentin to allow replacement resorption. Demineralized dentin block, as a three-dimensional scaffold, has been observed to be well incorporated with alveolar bone, with volume largely maintained and minor bone loss, after an average of 44 months of follow-up. Demineralized dentin block demonstrated significantly higher bone reformation, compared with undemineralized dentin block in a previous study. Graft biodegradation induced higher bone reformation according to previous studies. Similar to autologous block bone, demineralized dentin block and particulate dentin bone demonstrated graft biodegradation, which is considered an important factor in
CONCLUSIONS

Within the parameters of this study, both demineralized and undemineralized xenogenic dentin grafts in an onlay form revealed excellent bone regeneration over time. However, it can be concluded that demineralized and microperforated dentin block induced the fastest and highest bone formation compared with other dentin blocks. As an alternative to autogenous or allogenous bone graft, demineralized and microperforated dentin block can be recommended as an onlay block bone graft.

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