

**Vertical augmentation using autogenous
block bone and synthetic
hydroxyapatite block without fixation
on rabbit calvaria**

Soo-Yong Bae

The Graduate School

Yonsei University

Department of Dental Science

**Vertical augmentation using autogenous
block bone and synthetic
hydroxyapatite block without fixation
on rabbit calvaria**

Directed by Professor : Seong-Ho Choi

The Doctoral Dissertation Thesis
Submitted to the Department of Dental Science
and the Graduate School of Yonsei University
in partial fulfillment of the
requirements for the degree of
Ph.D. in Dental Science

Soo-Yong Bae

December 2012

This certifies that the Doctoral Dissertation
of Soo-Yong Bae is approved.

Thesis Supervisor : Seong-Ho Choi

Chang-Sung Kim

Ui-Won Jung

Byung-Ock Kim

Yeek Herr

The Graduate School

Yonsei University

December 2012

Table of Contents

List of figures	ii
List of tables	iii
Abstract (English)	iv
I. Introduction	1
II. Materials and Methods	5
1. Experimental animals.....	5
2. Fabrication of the HAB.....	5
3. Experimental procedure.....	6
4. Histologic evaluation.....	7
5. Radiographic evaluation.....	7
6. Histomorphometric evaluation.....	8
7. Statistical analysis	9
III. Results	10
1. Clinical findings.....	10
2. Radiographic findings: micro-CT.....	10
3. Histologic findings	11
3. Histomorphometric analysis	12
IV. Discussion	13
V. Acknowledgements	18
References	19
Tables	24
Figures legends	26
Figures	29
Abstract (In Korea)	34

List of Figures

Figure 1. Clinical photographs showing the study design	29
Figure 2. Schematic diagram of the grafted HAB, showing the histomorphometric analysis.	29
Figure 3. 3D-reconstructed images of the grafted materials at the different healing times.....	30
Figure 4. Histologic views of the ABB grafted area after 4 weeks (H&E stain).....	30
Figure 5. Histologic views of ABB grafted areas after 8 weeks (H&E stain).....	31
Figure 6. Histologic views of the HAB grafted area after 4 weeks (H&E stain).....	31
Figure 7. Histologic views of HAB grafted areas after 8 weeks (H&E stain).....	32
Figure 8. Residual bone area and remaining HA in cross-sectional view (%).	33

List of Tables

Table 1. Dimensions of the grafted autogenous block bone measured using micro CT (total volume) and histologic slides (area and height) (mean \pm SD; $n = 8$).....	24
Table 2. Dimensions of the grafted hydroxyapatite (HA) block (HAB) measured using micro CT (total volume) and histologic slides (area and height) (mean \pm SD; $n = 8$).....	24
Table 3. Relative dimensions of the grafted HAB (%; $n=8$).....	25

Abstract

Vertical augmentation using autogenous block bone and synthetic hydroxyapatite block without fixation on rabbit calvaria

Soo-Yong Bae, D.D.S.

Department of Dental Science

Graduate School, Yonsei University

(Directed by Professor Seong-Ho Choi, D.D.S., M.S.D., PhD.)

Purpose: The preferred material for bone augmentation beyond the envelope of skeletal bone is the block bone graft, due to its dimensional stability. We evaluated the necessity of rigid fixation for the block bone graft, and compared the bone regeneration and volume maintenance associated with grafting using a synthetic hydroxyapatite block (HAB) and an autogenous block bone (ABB) without rigid fixation on rabbit calvaria over two different periods.

Materials and methods: Cylinder-shaped synthetic HAB and ABB were

positioned without fixation on the rabbit calvarium ($n=16$). The animals were sacrificed at 4 and 8 weeks postoperatively, and the grafted materials were analyzed at each healing period using microcomputed tomography and histologic evaluation.

Results: Integration of the graft and the recipient bed was observed in all specimens, although minor dislocation of the graft materials from the original position was evident in some specimens (Six ABB and ten HAB samples). A tendency toward progressive bone resorption was observed in the grafted ABB but not in the grafted HAB, which maintained an intact appearance. In the HAB group, the area of new bone increased between 4 and 8 weeks postoperatively, but the difference was not statistically significant.

Conclusion: All of the nonfixed HAB was integrated into the recipient bed after both healing periods in rabbit calvaria. In spite of limited bone formation activity in comparison to ABB, HAB may be a favorable substitute osteoconductive bone material.

Keywords: animal experiments, bone regeneration, guided bone regeneration, histologic analysis, tissue physiology, rigid fixation

Vertical augmentation using autogenous block bone and synthetic hydroxyapatite block without fixation on rabbit calvaria

Soo-Yong Bae, D.D.S.

Department of Dental Science

Graduate School, Yonsei University

(Directed by Professor Seong-Ho Choi, D.D.S., M.S.D., PhD.)

I. Introduction

Various augmentation procedures have been introduced to treat a resorbed alveolar ridge, including guided bone regeneration with particulate or block-type materials, distraction osteogenesis, and ridge splitting(Fiorellini & Nevins 2003; McAllister & Haghghat 2007). Four factors are crucial to obtaining successful bone augmentation beyond the envelope of skeletal bone: primary closure, angiogenesis, space creation and maintenance, and stability of the blood clot(Wang & Boyapati 2006). Among these factors, the ability of space maintenance is highly emphasized, especially for large-sized defects, and hence the use of block bone grafts has been preferred due to their dimensional stability and resistance to deformation in unprotected areas that are

subject to compressive forces such as mastication(Barbosa et al. 2009).

There is a consensus in the literature that particulate or block-type autogenous bone is the gold standard for bone augmentation because of its osteogenic potential(Hoexter 2002; Jardini et al. 2005). However, autogenous block bone (ABB) has a tendency to be unpredictably resorbed and its acquisition is associated with a high morbidity in patients. Therefore, alloplastic block bone was introduced in an attempt to overcome these problems based on its favorable osteoconductive properties by providing the space and substratum for cellular and biologic events(Nasr et al. 1999). Particularly, the hydroxyapatite (HA) block (HAB) has received attention and is now widely used due to its excellent biocompatibility and osteointeractive properties(Nunes et al. 1997; Kurashina et al. 2002;Mangano et al. 2008). Furthermore, the HAB can be easily fabricated into any size or shape, and remains stable in normal physiological conditions since it is rapidly anchored to native bone via the deposition of bone mineral crystals directly onto the HA particles(Gauthier et al. 1998; Ducheyne & Qiu 1999; Karageorgiou & Kaplan 2005).

The need for a rigid fixation with screw to ensure good mechanical stability of the block bone and the union with the recipient bed during the initial healing period has been demonstrated in various literatures(Phillips & Rahn 1988, 1990; Cha et al. 2011; Oh et al. 2011). Phillips and Rahn(Phillips & Rahn 1988, 1990) showed that the resorption of rigidly screw fixed onlay bone grafts in sheep was less than for grafts that were not fixed, and they postulated that this finding was attributable mainly to movement between the graft and the recipient bed. LaTrenta et al.(LaTrenta et al.

1989) showed that nonrigidly wire fixed endochondral bone grafts in a dog model predominately healed with a fibrous union, from which they postulated that rigid screw fixation could induce rapid revascularization, increasing the contact area between the graft and the recipient site, thus providing an ideal environment for “creeping substitution.” Moreover, it was proposed that movement of the grafted materials during the early stages of wound healing could lead to failure of osseointegration by differentiation of mesenchymal cells into fibroblasts instead of osteoblasts(Burchardt 1983).

In order to provide rigid fixation between graft materials and the recipient bed, the titanium bone-fixation screw has typically been used, but this has some potential limitations such as additional patient discomfort due to the required second surgery, the wide flap required for removing the screw (which increases the risk of resorption of the newly formed bone), radiographic artifacts, corrosion, allergic reaction, and cold sensitivity(Chacon et al. 2004). Moreover, brittle graft materials could break during the screw-fixation process. Therefore, the alternative method to circumvent the fixation of the grafted block bone has been long sought.

Recent studies have shown predictable bony union with nonrigid fixation techniques(De Marco et al. 2005; Jardini et al. 2005). Lin et al.(Lin et al. 1990) reported the possibility of vertical augmentation without fixation in a rabbit model. In areas where high shear and torsion are expected, graft volume was decreased in the nonfixed group than in the rigidly fixed group, but no differences in graft volume retention or graft survival were observed between the fixed and nonfixed conditions

in a low-motion region.

The objectives of this study were to characterize the bone regeneration and maintenance of volume on rabbit calvaria using the synthetic HAB without rigid fixation, and to compare the healing pattern with the nonfixed ABB over two different healing periods of 4 and 8 weeks.

II. Material and methods

1. Experimental animals

A total of 16 male New Zealand white rabbits (3.0–3.5 kg, $n=8$ at 4 weeks, $n=8$ at 8 weeks) were used in this study. The animals were kept under standard laboratory conditions, fed a standard diet, and raised in separate cages. The animal selection, management, surgical protocol, and preparation followed routines approved by the Institutional Animal Care and Use Committee, Yonsei Medical Center, Seoul, Korea(certification #2010-070).

2. Fabrication of the HAB

A simple porous scaffold was produced according to previously published methods(Jang et al. 2011). Briefly, the sol-gel process was used to prepare HA nanoparticles. Selected precursors of the HA sol were $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ (99%; Sigma-Aldrich, St. Louis, MO, USA) and $(\text{OC}_2\text{H}_5)_3\text{P}$ (97%; Sigma-Aldrich). The Ca precursor was dissolved in methyl alcohol at a stoichiometric Ca:P ratio of 1.67. The solution was dehydrated at 180°C after solvent evaporation, and the solution was refluxed in methyl alcohol in an Ar atmosphere. The prehydrolysis of the P precursor was carried out over 10 h; HCl and H_2O were added as catalysts before the reaction with the Ca precursor. The two precursors were reacted to prepare the HA solution,

which was subsequently dried at 950°C. The finished scaffold was an 8-mm-diameter and 3-mm-high disk with a porosity, specific surface, strut thickness, and strut spacing of 81.55%, 31.25 mm²/mm³, 140.14 μm, and 523.12 μm, respectively.

3. Experimental procedure

The animals were anesthetized with an intramuscular injection of a mixture of ketamine hydrochloride (Ketalar, Yuhan, Seoul, Korea) and xylazine (Rompun, Bayer Korea, Seoul, Korea). The surgical sites were shaved and then swabbed with alcohol and povidone iodine, and then a local anesthetic (2% lidocaine; lidocaine HCl, Huons, Seoul, Korea) was administered. An incision was made along the sagittal midline from the frontal bone to the occipital bone. A full-thickness flap was elevated to expose the cranial bone. Two standardized circular grooves that incompletely penetrated the skull were prepared as the recipient beds in the bone on each side of the midline using a trephine drill (diameter, 8 mm) under cool-saline irrigation. Four cortical perforations within the circle were then drilled to induce bleeding from the marrow space with a 1.5-mm diameter carbide bur. ABB was obtained from the posterior rabbit calvaria using a trephine bur of the same diameter, and then the synthetic HAB and the ABB were positioned on the prepared recipient beds without fixation (Fig. 1). The soft tissues were repositioned and then sutured layer by layer with a resorbable suture material (4-0 Vicryl, Ethicon, Somerville, NJ, USA) to achieve primary closure. The stitches were removed after 10 days. The animals were

sacrificed at 4 or 8 weeks postoperatively, and block sections of the graft sites were collected and prepared for radiographic and histomorphometric evaluation.

4. Histologic evaluation

The harvested block sections were fixed in 10% buffered formalin and decalcified with 5% formic acid for 14 days. Paraffin wax blocks were made and sectioned in the mesiodistal direction at a thickness of 5 μm . The most-central sections from each block were stained with hematoxylin and eosin. General histological findings were observed under a microscope (Olympus BX41, Tokyo, Japan).

5. Radiographic evaluation

The fixed block specimens were scanned using a microcomputed tomography (micro-CT) system (SkyScans1072, SkyScan, Aartselaar, Belgium) at a resolution of 18 μm (100 kV and 100 μA). The scanned sets of data were processed in DICOM format, and OnDemand 3D software (Cybermed, Seoul, Korea) was used to reconstruct the area of interest. The overall dimensional topography of the grafts and recipient beds was visualized with a 3D-reconstructed image, and the residual volume (in mm^3) of the block graft was measured in 3D-reconstructed images.

6. Histomorphometric evaluation

The histologic and radiographic analysis was performed by one experienced researcher (S.Y.B.). An automated image analysis system (Image Pro Plus, Media Cybernetics, SilverSpring, MD, USA) was used for the histomorphometric analysis. The following parameters were measured (Fig. 2):

- ***ABB graft***

- i. Augmented area (mm^2): the area of all tissues beyond the cranial vault.
- ii. Density of augmented bone: percentage of trabeculae relative to the augmented area (%).

- ***HAB graft***

- i. Augmented area (mm^2): the area of all tissues beyond the cranial vault.
- ii. New bone (mm^2 ; %): only the area with newly formed mineralized bone (mm^2) and the percentage of newly formed mineralized bone area relative to the augmented area (%).
- iii. Residual materials (mm^2 ; %): the area of remaining grafted material (mm^2) and the percentage of remaining grafted material area relative to the augmented area (%).
- iv. Fibrovascular tissue (%): percentage of vascular tissue and connective tissue area relative to the augmented area (%).

In both groups, the vertical height (mm) of the augmented area was measured at

1 mm from the lateral and medial boundary edges and the center of augmented material.

7. Statistical analysis

Mean and standard deviation values were calculated for each group from the measurements taken from the central section of each of the grafted materials and the 3D micro-CT data. Statistical differences were determined by analysis of variance and the *post-hoc t*-test for multiple comparisons, and by the unpaired *t*-test for comparisons between two independent groups using a statistical software program SPSS (ver. 15.0, SPSS, Chicago, IL, USA). The level of statistical significance was set at $P < 0.05$.

III. Results

1. Clinical findings

Surgical wound healing was uneventful throughout the experimental period. No complications were observed, including wound dehiscence, severe swelling, or bleeding.

2. Radiographic findings: micro-CT

A shift in the location of the integrated graft materials was clearly observed in six ABB specimens (three at 4 weeks and three at 8 weeks) and ten HAB specimens (five at 4 weeks and five at 8 weeks). For the ABB group, distinctive external resorption was observed at 4 weeks of healing, and the bone resorption was lowest at the central area and highest at the boundary area, resulting in a dome-shaped appearance; there was minimal resorption in the HAB group at this stage. External resorption became much evident at 8 weeks of healing at the boundary area in the grafted ABB, while there was still minimal resorption in the grafted HAB and its morphology appeared intact, maintaining its original shape. The total volumes of grafted ABB and HAB are measured using micro CT analysis and presented in Tables 1 and 2. The residual volume of ABB was 40.8 mm^3 at 4 weeks and 38.9 mm^3 at 8 weeks; the difference was not statistically significant. The residual volume of HAB at these two time points

was 91.7 mm³ and 94.5 mm³, respectively (also not significantly different; Fig. 3).

3. Histologic findings

- ***ABB group***

Histologic integration of the graft and the recipient bed was observed in all groups at both healing periods. At 4 weeks, the graft–recipient bed interface exhibited a bony bridge connection after intense remodeling in the area adjacent to the graft; however, three of the specimens at 4 weeks and one at 8 weeks contained small areas of connective tissue partially interposed between the graft and the recipient bed. The block presented an absorbed appearance at the lateral borders and the upper surface, displaying a rounded rectangular shape with an irregular border, and increased adipose tissue invaginations were observed in the intramedullary space (Fig. 4). At 8 weeks, the resorption processes appeared to have continued, resulting in a dome-shaped appearance. The remodeling in the area adjacent to the graft had progressed further, so it was difficult to distinguish the interface between the grafted bone and the recipient bed. The maturation of the grafted block bone appeared to have progressed further than at 4 weeks, and a large amount of adipose tissue was observed in the intramedullary space (Fig. 5).

- ***HAB graft***

At 4 and 8 weeks, the grafted HAB materials were well maintained under the

connective tissue layer and there was little or no change in the general outline at both time points. Intimate contact between graft materials and the recipient bed at the interface surface—indicating bony union—was observed in both groups. The HAB appeared to be infiltrated by newly formed bone, without signs of necrosis or osteolysis, and direct contact was observed between the newly formed bone and the graft materials. Newly formed bone was observed along the interface between the recipient bone and the block, and numerous vascular tissues were observed within the remaining graft materials. Resorption of graft materials was barely noticeable over the healing period (Figs. 6 and 7).

4. Histomorphometric analysis

The results of the histomorphometric analysis are presented in Tables 1–3 and Fig. 8. The total area of both the ABB and HAB grafts appeared to have reduced between 4 and 8 weeks postoperatively, and the area of new bone regeneration with the HAB graft appeared to be increased at 8 weeks than at 4 weeks; however, neither of these differences was statistically significant. With regard to the height of the augmented area of the ABB and HAB grafts, the central area was slightly greater than the medial and lateral areas 1 mm from the boundary edge.

IV. Discussion

We have evaluated the effectiveness of synthetic HAB for bone regeneration and volume maintenance, and compared it with ABB, under the nonfixed condition in rabbit calvaria. The rabbit calvarial model has been used as a bone-defect model for non-load-bearing bone and for evaluating various biomaterials and regenerative techniques due to its resemblance to human mandibular bone, which has a poor blood supply and limited bone marrow (Frame 1980; Sohn et al. 2010). In the present study we evaluated the outcome of these graft materials at 4 weeks postoperatively, which represents the early phase of the healing response, and measured properties such as the stability of the materials and host reactions; at 8 weeks postoperatively, which represents the late healing response, we measured processes such as bone incorporation, resorption of materials, bone remodeling, and the amount of bone regeneration (Sohn et al. 2010).

Regarding the rigid fixation of block bone grafts, numerous literatures demonstrate the need for a rigid fixation that can avoid fracture at the graft–bed interface since the mechanical stability of the grafted materials is essential to minimize micromovement during the initial healing phase. Previous studies have demonstrated that rigid screw fixation reduces resorption and increases the success rate of the ABB graft (Phillips & Rahn 1988, 1990), while a lack of rigid fixation induces predominantly fibrous union with the endochondral ABB graft and recipient

bed(LaTrenta et al. 1989). However, some studies found successful graft volume retention and survival without rigid screw fixation (Lin et al. 1990; Jardini et al. 2005). In the present study both the nonfixed HAB and ABB successfully fused onto the recipient bed although the location of the graft material had clearly shifted before integration on the recipient bed in six of the ABB specimens (three at 4 weeks and three at 8 weeks) and ten of the HAB specimens (five at 4 weeks and five at 8 weeks). Generally, the rabbit calvarium is considered to be a pressure- and movement-free region, but some of the typical behaviors of rabbits, such as grooming, can have an undesirable effect on graft material stability. Nevertheless, successful integration between the recipient bed and bone graft was observed at 4 and 8 weeks postoperatively in all groups. Interestingly, the number of clearly shifted graft materials was greater for HAB than for ABB grafts. We postulate that the bottom surface of the ABB obtained from rabbit calvaria was convex in shape, thus increasing the contact surface over the calvaria and enhancing the stabilization of the grafted block. Meanwhile, the manufactured HAB had a flat surface morphology, which did not match the convex shape of the recipient bed resulted in increased mobility in the early stage of healing.

Histologic analysis was performed only on representative central cross-sectional views, and was not sufficient to observe 3D changes of wound healing events. Therefore, 3D micro-CT was used in this study to observe the overall healing pattern of the graft materials. Definite external bone resorption was observed in micro-CT images at 4 weeks for the ABB grafts, and continued resorption was noted at 8 weeks,

although the difference in mean volume between the two time points was not significant. Especially, without exclusive membrane, autogenous block bone graft underwent extensive resorption in early healing period because of revascularization from surrounding connective tissue which induce peripheral osteoclastic resorption, and flap tension exerted on the external edge(Jardini 2005). Meanwhile, in the HAB graft there was no sign of resorption and the graft appeared to remain intact.

In general, the height of obtained bone was lower at the medial and lateral positions than at the central position in both the ABB and HAB groups, although the difference was not statistically significant. According to our histologic analysis and 3D micro-CT evaluation, the tendency toward the progression of bone resorption in the grafted ABB material implies that the augmented ABB could be unpredictably resorbed, while the volume of the synthetic HAB graft was maintained throughout the 8-week postoperative period. The area of new bone in the HAB group tended to increase between 4 and 8 weeks, although again the difference did not reach statistical significance. Furthermore, the total area of new bone and the remaining grafted materials in the HAB group was lower than the mineralized area of the augmented ABB at both healing periods; it nevertheless appeared to be sufficient to achieve complete integration. New bone had infiltrated to two-thirds of the overall height in three specimens at 4 weeks and four specimens at 8 weeks, suggesting that HAB has substantial potential in vertical bone augmentation procedures even without fixation.

Several long-term studies have demonstrated that HA can be used in the treatment of atrophic ridges as a predictable and stable biomaterial(el Deeb et al. 1991;

Mercier et al. 1996;Proussaefs et al. 2002). However, the success of HA is limited primarily due to its slow degradation rate(Gao et al. 2004). The quality and quantity of newly formed bone can only be maximized when the degradation rate is similar to the rate of bone formation(Jammet et al. 1994; Leeuwenburgh et al. 2001). It has generally been believed that HA is nonresorbable; however, different types of HA have different resorption rates(Donath et al. 1987; Goto et al. 2001), and the resorption rate and mechanical strength of HA can be controlled by varying its porosity, the sintering method used in its production, and the composition of the graft material itself(Donath et al. 1987; Jammet et al. 1994; Goto et al. 2001; Leeuwenburgh et al. 2001). In vertical bone augmentation beyond the bony envelope, such as in the present study, it appears that the slow degradation rate is advantageous in that it allows the graft material to maintain its volume and provide space. Furthermore, the authors have previously shown that HA scaffold can be applied to tissue engineering technology by using mesenchymal stem cells or various growth factors(Jang et al. 2012; Kim et al. 2011; Park et al. 2011). Therefore, future studies might consider the application of growth factor or stem cells in HAB for vertical bone augmentation without fixation.

The defect-driven customized block bone fabrication for various bone defects using computed tomography imaging and CAD/CAM system have been tried(Ciocca et al. 2009, Sun et al. 2004). If this trial is widely used, a block bone graft material for the irregular bone defect of recipient site can be made and applied. Its fitness of customized bone graft into the defect increase wound stability in early healing phase

and more desirable result can be achieved. However, there have been a few studies in such trial.

In conclusion, within the limitations of this study, the use of HAB grafts appeared to be beneficial for vertical bone augmentation beyond the skeletal bone envelope and the maintenance of space in the rabbit calvarial model, despite the lack of fixation. In spite of the limited bone-forming activity relative to ABB, HAB can be used as a favorable osteoconductive bone substitute material.

V. Acknowledgements

All authors declare no conflict of interest. This study was supported by a grant of the Korea Health technology R&D Project, Ministry of Health & Welfare, Republic of Korea. (101578)

References

- Barbosa, D.Z., de Assis, W.F., Shirato, F.B., Moura, C.C., Silva, C.J. & Dechichi, P. (2009) Autogenous bone graft with or without perforation of the receptor bed: histologic study in rabbit calvaria. *Int J Oral Maxillofac Implants***24**: 463-468.
- Burchardt, H. (1983) The biology of bone graft repair. *Clin Orthop Relat Res*: 28-42.
- Cha, J.K., Kim, C.S., Choi, S.H., Cho, K.S., Chai, J.K. & Jung, U.W. (2012) The influence of perforating the autogenous block bone and the recipient bed in dogs. Part II: histologic analysis. *Clinical oral implants research* 23(8):987-992.
- Chacon, G.E., Ellis, J.P., Kalmar, J.R. & McGlumphy, E.A. (2004) Using resorbable screws for fixation of cortical onlay bone grafts: an in vivo study in rabbits. *J Oral Maxillofac Surg***62**: 1396-1402.
- Ciocca, L., De Crescenzo, F., Fantini, M. & Scotti, R. (2009) Cad/cam and rapid prototyped scaffold construction for bone regenerative medicine and surgical transfer of virtual planning: A pilot study. *Computerized medical imaging and graphics* 33: 58-62.
- De Marco, A.C., Jardini, M.A. & Lima, L.P. (2005) Revascularization of autogenous block grafts with or without an e-PTFE membrane. *Int J Oral Maxillofac Implants***20**: 867-874.
- Donath, K., Rohrer, M.D. & Beck-Mannagetta, J. (1987) A histologic evaluation of a mandibular cross section one year after augmentation with hydroxyapatite particles. *Oral Surg Oral Med Oral Pathol***63**: 651-655.

- Ducheyne, P. & Qiu, Q. (1999) Bioactive ceramics: the effect of surface reactivity on bone formation and bone cell function. *Biomaterials***20**: 2287-2303.
- el Deeb, M., Tompach, P.C., Morstad, A.T. & Kwon, P. (1991) Long-term follow-up of the use of nonporous hydroxyapatite for augmentation of the alveolar ridge. *J Oral Maxillofac Surg***49**: 257-261.
- Fiorellini, J.P. & Nevins, M.L. (2003) Localized ridge augmentation/preservation. A systematic review. *Ann Periodontol***8**: 321-327.
- Frame, J.W. (1980) A convenient animal model for testing bone substitute materials. *J Oral Surg***38**: 176-180.
- Gao, H., Tan, T. & Wang, D. (2004) Effect of composition on the release kinetics of phosphate controlled release glasses in aqueous medium. *J Control Release***96**: 21-28.
- Gauthier, O., Bouler, J.M., Aguado, E., Pilet, P. & Daculsi, G. (1998) Macroporous biphasic calcium phosphate ceramics: influence of macropore diameter and macroporosity percentage on bone ingrowth. *Biomaterials***19**: 133-139.
- Goto, T., Kojima, T., Iijima, T., Yokokura, S., Kawano, H., Yamamoto, A. & Matsuda, K. (2001) Resorption of synthetic porous hydroxyapatite and replacement by newly formed bone. *J Orthop Sci***6**: 444-447.
- Hoexter, D.L. (2002) Bone regeneration graft materials. *J Oral Implantol***28**: 290-294.
- Jammet, P., Souyris, F., Baldet, P., Bonnel, F. & Huguët, M. (1994) The effect of different porosities in coral implants: an experimental study. *J Craniomaxillofac Surg***22**: 103-108.

- Jang, J.W., Yun, J.H., Lee, K.I., Jung, U.W., Kim, C.S., Choi, S.H. & Cho, K.S. (2012) Osteoinductive activity of biphasic calcium phosphate with different rhBMP-2 doses in rats. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod***113**:480-487
- Jang, Y., Jung, I., Park, J., Jung, U., Kim, C., Lee, Y. & Choi, S. (2011) Effect of seeding using an avidin-biotin binding system on the attachment of periodontal ligament fibroblasts to nanohydroxyapatite scaffolds: three-dimensional culture. *Journal of periodontal & implant science***41**: 73-78.
- Jardini, M.A., De Marco, A.C. & Lima, L.A. (2005) Early healing pattern of autogenous bone grafts with and without e-PTFE membranes: a histomorphometric study in rats. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod***100**: 666-673.
- Karageorgiou, V. & Kaplan, D. (2005) Porosity of 3D biomaterial scaffolds and osteogenesis. *Biomaterials***26**: 5474-5491.
- Kim, J.W., Choi, K.H., Yun, J.H., Jung, U.W., Kim, C.S., Choi, S.H. & Cho, K.S. (2011) Bone formation of block and particulated biphasic calcium phosphate lyophilized with Escherichia coli-derived recombinant human bone morphogenetic protein 2 in rat calvarial defects. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod***112**: 298-306.
- Kurashina, K., Kurita, H., Wu, Q., Ohtsuka, A. & Kobayashi, H. (2002) Ectopic osteogenesis with biphasic ceramics of hydroxyapatite and tricalcium phosphate in rabbits. *Biomaterials***23**: 407-412.
- LaTrenta, G.S., McCarthy, J.G., Breitbart, A.S., May, M. & Sissons, H.A. (1989) The

- role of rigid skeletal fixation in bone-graft augmentation of the craniofacial skeleton. *Plast Reconstr Surg***84**: 578-588.
- Leeuwenburgh, S., Layrolle, P., Barrere, F., de Bruijn, J., Schoonman, J., van Blitterswijk, C.A. & de Groot, K. (2001) Osteoclastic resorption of biomimetic calcium phosphate coatings in vitro. *J Biomed Mater Res***56**: 208-215.
- Lin, K.Y., Bartlett, S.P., Yaremchuk, M.J., Fallon, M., Grossman, R.F. & Whitaker, L.A. (1990) The effect of rigid fixation on the survival of onlay bone grafts: an experimental study. *Plast Reconstr Surg***86**: 449-456.
- Mangano, C., Piattelli, A., Perrotti, V. & Iezzi, G. (2008) Dense hydroxyapatite inserted into postextraction sockets: a histologic and histomorphometric 20-year case report. *J Periodontol***79**: 929-933.
- McAllister, B.S. & Haghghat, K. (2007) Bone augmentation techniques. *J Periodontol***78**: 377-396.
- Mercier, P., Bellavance, F., Cholewa, J. & Djokovic, S. (1996) Long-term stability of atrophic ridges reconstructed with hydroxylapatite: a prospective study. *J Oral Maxillofac Surg***54**: 960-968; discussion 968-969.
- Nasr, H.F., Aichelmann-Reidy, M.E. & Yukna, R.A. (1999) Bone and bone substitutes. *Periodontol 2000***19**: 74-86.
- Nunes, C.R., Simske, S.J., Sachdeva, R. & Wolford, L.M. (1997) Long-term ingrowth and apposition of porous hydroxylapatite implants. *J Biomed Mater Res***36**: 560-563.
- Oh, K.C., Cha, J.K., Kim, C.S., Choi, S.H., Chai, J.K. & Jung, U.W. (2011) The

- influence of perforating the autogenous block bone and the recipient bed in dogs.
Part I: a radiographic analysis. *Clinical oral implants research***22**: 1298-1302.
- Park, J.C., So, S.S., Jung, I.H., Yun, J.H., Choi, S.H., Cho, K.S. & Kim, C.S. (2011)
Induction of bone formation by Escherichia coli-expressed recombinant human
bone morphogenetic protein-2 using block-type macroporous biphasic calcium
phosphate in orthotopic and ectopic rat models. *J Periodontal Res***46**: 682-690.
- Phillips, J.H. & Rahn, B.A. (1988) Fixation effects on membranous and endochondral
onlay bone-graft resorption. *Plast Reconstr Surg***82**: 872-877.
- Phillips, J.H. & Rahn, B.A. (1990) Fixation effects on membranous and endochondral
onlay bone graft revascularization and bone deposition. *Plast Reconstr Surg***85**:
891-897.
- Proussaefs, P., Lozada, J., Valencia, G. & Rohrer, M.D. (2002) Histologic evaluation
of a hydroxyapatite onlay bone graft retrieved after 9 years: a clinical report. *J
Prosthet Dent***87**: 481-484.
- Sohn, J.Y., Park, J.C., Um, Y.J., Jung, U.W., Kim, C.S., Cho, K.S. & Choi, S.H. (2010)
Spontaneous healing capacity of rabbit cranial defects of various sizes. *J
Periodontal Implant Sci***40**: 180-187.
- Sun, W., Darling, A., Starly, B. & Nam, J. (2004) Computer-aided tissue engineering:
Overview, scope and challenges. *Biotechnology and applied biochemistry***39**: 29-
47.
- Wang, H.L. & Boyapati, L. (2006) "PASS" principles for predictable bone
regeneration. *Implant Dent***15**: 8-17.

Tables

Table 1. Dimensions of the grafted autogenous block bone measured using micro CT (total volume) and histologic slides (area and height) (mean \pm SD; $n=8$)

Healing time	Total volume (mm ³)	Total area (mm ²)	Density(%)	Grafted height (mm)		
				Medial	Central	Lateral
4 weeks	40.08 \pm 6.63	9.95 \pm 1.96	44.65 \pm 7.14	1.54 \pm 0.50	1.78 \pm 0.32	1.28 \pm 0.39
8 weeks	38.9 \pm 12.51	8.95 \pm 0.95	44.73 \pm 14.56	1.36 \pm 0.28	1.68 \pm 0.26	1.14 \pm 0.28

No significant differences between the two healing periods ($P>0.05$).

Table 2. Dimensions of the grafted hydroxyapatite (HA) block (HAB) measured using micro CT (total volume) and histologic slides (area and height) (mean \pm SD; $n=8$)

Healing time	Total volume (mm ³)	Total area (mm ²)	New bone area(mm ²)	HA area (mm ²)	Grafted height(mm)		
					Medial	Central	Lateral
4 weeks	91.70 \pm 15.00	18.39 \pm 2.48	1.13 \pm 0.72	2.93 \pm 0.68	2.21 \pm 0.48	2.65 \pm 0.43	2.41 \pm 0.44
8 weeks	94.47 \pm 12.44	16.66 \pm 3.18	1.68 \pm 0.89	2.76 \pm 0.50	2.20 \pm 0.63	2.38 \pm 0.43	2.33 \pm 0.61

No significant differences between the two healing periods ($P>0.05$).

Table 3. Relative dimensions of the grafted HAB (%; n=8)

Healing time	New bone area	HA area	Fibrovascular area
4 weeks	6.34±4.55	16.00±3.40	77.66±7.20
8 weeks	10.49±5.80	16.98±3.90	72.53±5.96

No significant differences between the two healing periods ($P>0.05$).

Figure legends

Fig. 1. Clinical photographs showing the study design. (a) Trephine drills were used to mark the recipient beds for the autogenous bone block (ABB) and hydroxyapatite block (HAB). The arrow indicates the donor site for the ABB. (b) ABB and HAB were applied on the recipient beds, and neither fixation nor a membrane was used.

Fig. 2. Schematic diagram of the grafted HAB, showing the histomorphometric analysis. The total augmented area includes all tissue, consisting of newly formed bone, fibrovascular tissue, and residual bone substitute. Arrows indicate the three positions of height measurement: medial position at 1 mm from the boundary edge, lateral position at 1 mm from the boundary edge, and the center position.

Fig. 3. 3D-reconstructed images of the grafted materials at the different healing times: (a) ABB (green color) at 4 weeks, (b) HAB (purple color) at 4 weeks, (c) ABB (green color) at 8 weeks, (d) HAB (purple color) at 8 weeks, (e) cross-sectional view of ABB at 4 weeks, (f) cross-sectional view of HAB at 4 weeks, (g) cross-sectional view of ABB at 8 weeks, and (h) cross-sectional view of HAB at 8 weeks.

Fig. 4. Histologic views of the ABB grafted area after 4 weeks (H&E stain): (a) General outline of grafted material over the residual bone ($\times 40$). (b) Fusion of grafted ABB onto the residual bone is observed with the formation of bone marrow space. (c) Relative resorption of ABB is observed along the upper boarder of the ABB. Inset boxes in lower magnification ($\times 40$) represent the corresponding area in higher magnification ($\times 200$) and corresponding figures are labelled accordingly. RB, residual bone; GB, grafted bone; NB, new bone.

Fig. 5. Histologic views of ABB grafted areas after 8 weeks (H&E stain): (a) Relatively increased maturation of the fused area between ABB and RB is observed in comparison to 4 weeks ($\times 40$). (b) ABB has completely fused onto the residual bone ($\times 200$). (c) Outline of ABB has more decreased ($\times 200$). RB, residual bone; GB, grafted bone.

Fig. 6. Histologic views of the HAB grafted area after 4 weeks (H&E stain): (a) General outline of grafted material over the residual bone ($\times 40$). Residual materials are observed within the outline of grafted HAB. (b) Moderate amount of new bone growth into the HAB was observed over the residual bone. (c) Highly lamellated new bone growth is observed among the HAB graft. Inset boxes in lower magnification ($\times 40$) represent the corresponding area in higher magnification ($\times 200$) and corresponding figures are labelled accordingly. RB, residual bone; NB, new bone; HA, remaining HA.

Fig. 7. Histologic views of HAB grafted areas after 8 weeks (H&E stain): (a) Residual materials are still observed over the new bone ingrowth ($\times 40$). The general outline is highly preserved over the healing period. (b) New bone has grown into the space of HAB and fully matured showing highly lamellated pattern ($\times 200$). (c) Residual materials are still observed among the new bone growth ($\times 200$). RB, residual bone; GB, grafted bone; NB, new bone; HA, remaining HA.

Fig. 8. Residual bone area and remaining HA in cross-sectional view (%). There was no significant difference between 4 and 8 weeks ($P > 0.05$).

Figures

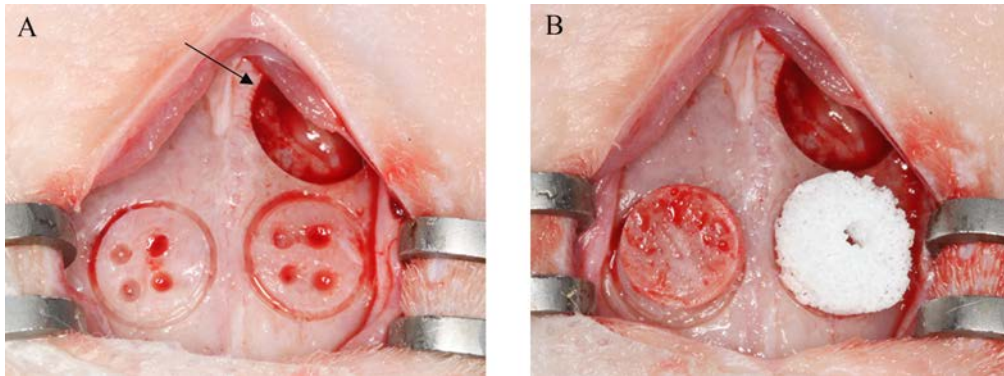


Figure 1

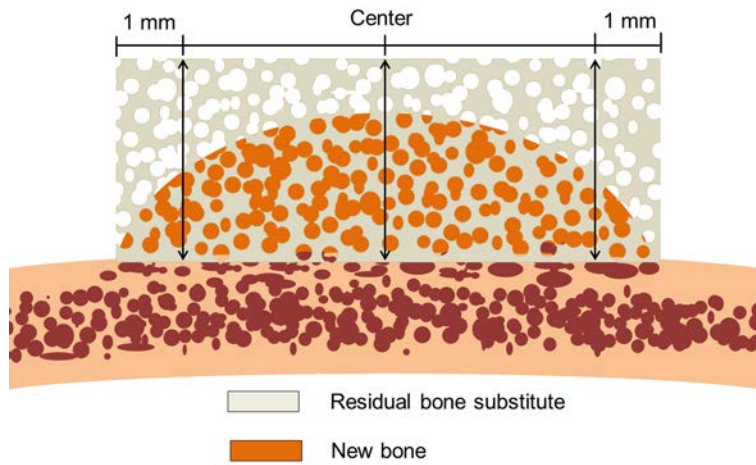


Figure2

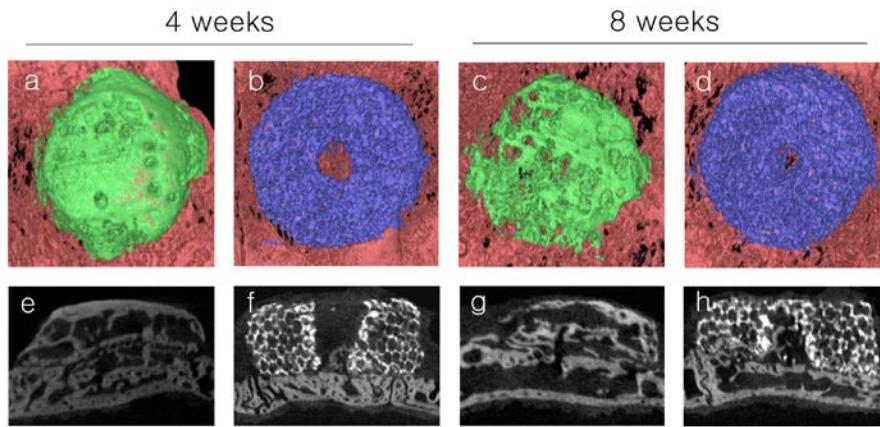


Figure 3

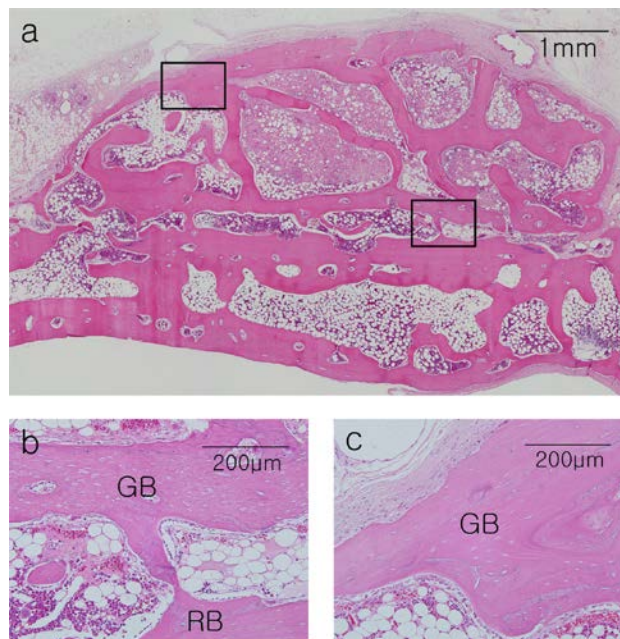


Figure 4

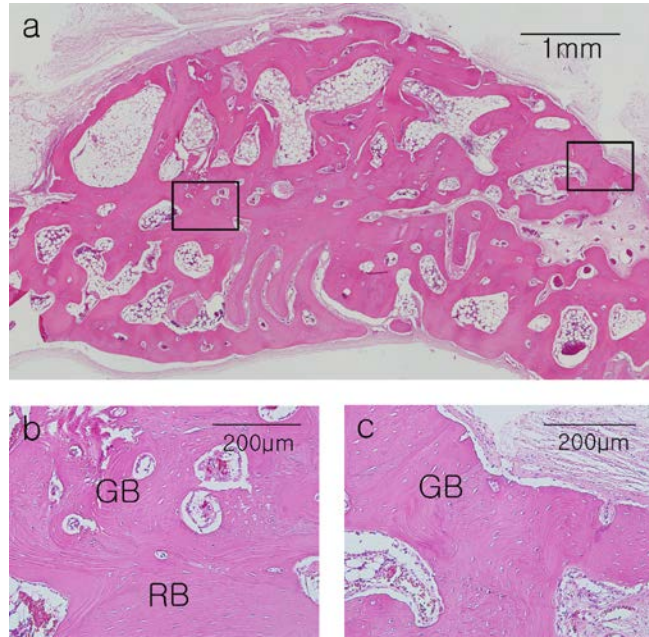


Figure 5

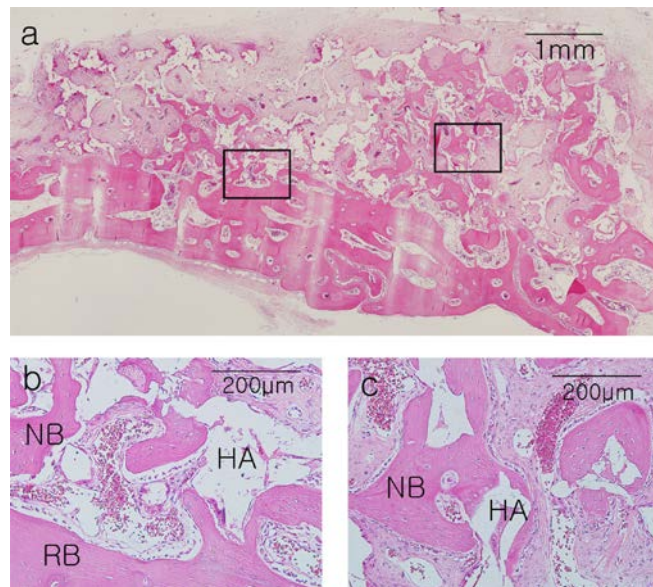


Figure 6

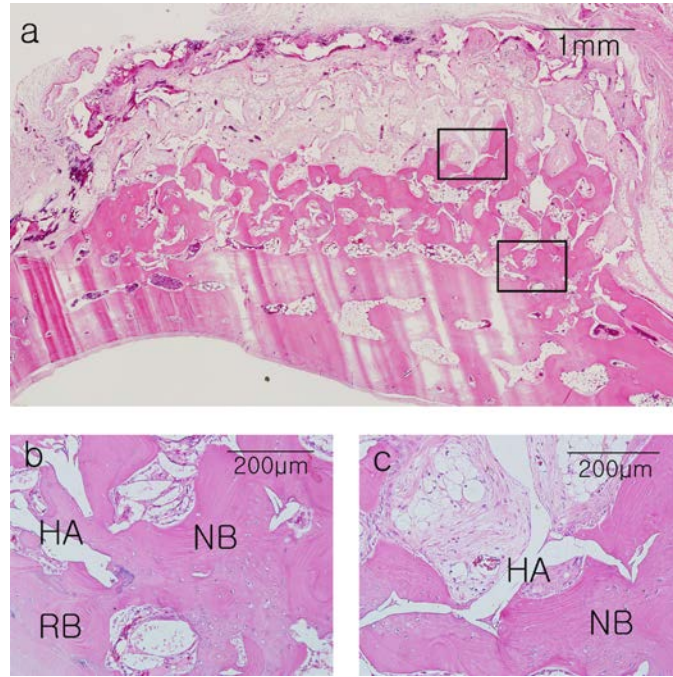


Figure 7

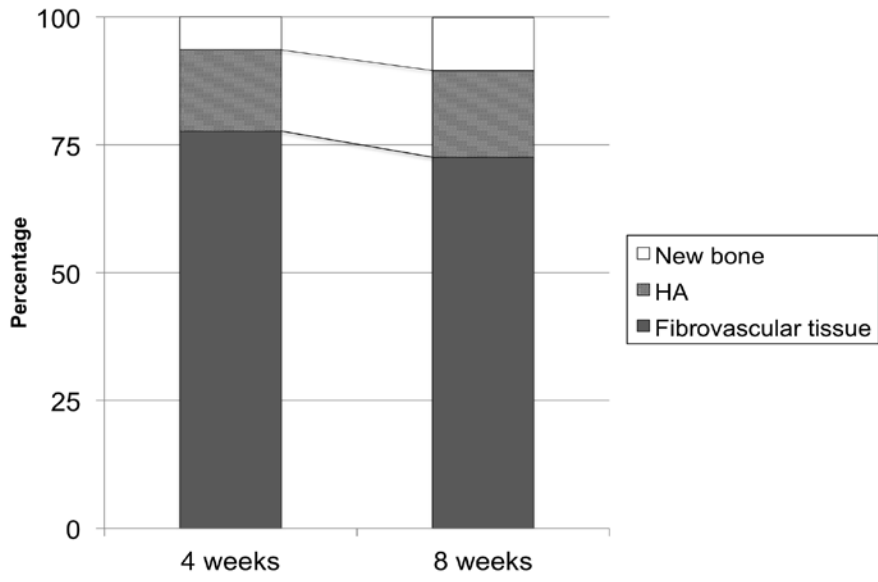


Figure 8

국문요약

백서 두개골에서 비고정 방법을 통해 자가골 블록과 합성 Hydroxyapatite

블록을 이용한 수직적 골 형성

연세대학교 대학원 치의학과

(지도 최성호 교수)

배 수 용

골의 기존 경계를 넘어서 새로운 골을 형성 하기 위해서는 블록 형태의 골 이식재가 형태학적인 안정성 때문에 더욱 선호 된다. 자가골은 그 자체의 골 형성 능력 때문에 가장 좋은 선택으로 고려 되어 지고 있으나 이식 후 예상할 수 없는 흡수 경향을 보이고 그 채취 과정에서 환자에게 적지 않은 부작용을 발생 시킬 수 있기 때문에 다른 대체 재료들이 고려 되어 왔고 Hydroxyapatite는 생체친화성과 골전도 능력, 제작의 용이성으로

인해 많은 관심을 받아 왔다. 이식편의 강성 고정은 초기 치유 기간의 물리적인 안전성을 이루기 위해 필요 하다고 많은 연구에서 보고 하고 있다. 이에 본 연구는 블록 형태의 골 이식재를 이용 할 때 강성 고정의 필요 유무에 대한 평가를 시행 하였고 서로 다른 두 기간 동안 블록 형태의 자가골과 합성 Hydroxyapatite 블록을 고정 없이 백서 두개골에 이식 했을 때 이식편의 골 재생 형태와 부피 유지 능력을 비교 하였다.

본 연구에서는 원통 형태의 합성 Hydroxyapatite와 자가골이 16마리 백서 두개골에 고정 없이 이식되었다. 실험 동물은 이식편을 위치 시킨 4주와 8주 후에 희생되었으며 이식편은 각각의 시점에서 단층 촬영과 조직학적 분석에 의해 평가 되었다.

실험 결과 모든 실험 군에서 이식 편과 수여부의 유합이 일어났으나 초기 이식 부위에서 이동된 위치에서의 유합이 6개의 자가골 실험군, 10개의 Hydroxyapatite 실험군에서 관찰되었다. 자가골 이식편은 실험 기간에 따라 지속적인 골의 흡수가 관찰되었지만 Hydroxyapatite 그룹은 초기의 온전한 형태를 유지하고 있음이 관찰되었다. Hydroxyapatite

그룹에서는 4주에서 8주로 이행 될수록 신생골의 증가가 관찰되었지만 통계학적으로 유의성 있는 차이를 보이지는 못하였다.

결론적으로, Hydroxyapatite 블록은 고정 방법을 사용하지 않고서도 이식부위에 잘 유합 되었고 자가골 블록에 비해 제한적인 골 형성 능력을 보였지만 바람직한 골 전도 능력을 보이는 골 이식재로 고려 될 수 있다.

핵심 되는 말: 동물 실험, 골 재생, 골 유도 재생, 조직 분석, 조직 생리,

강성 고정