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<table>
<thead>
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| 投稿内容      | 金沢大学学術情報リポジトリのコンテンツに投稿される投稿論文の一部を掲載

金沢大学学術情報リポジトリ(金沢大学)
An experimental study of use of absorbable plate in combination with self-setting 
\(\alpha\)-tricalcium phosphate for orthognathic surgery

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An experimental study of use of absorbable plate in combination with self-setting α-tricalcium phosphate for orthognathic surgery

Abstract

Purpose: The purpose of this study was to histologically and immuno-histochemically evaluate bone formation using both of self-setting α-tricalcium phosphate (α-TCP; BIOPEX®) and absorbable plate (Super FIXSORB®-MX) in rabbit cranium bone.

Study design: Adult male Japanese white rabbits (n=16, 12-16 weeks, 2.5-3.0 kg) were used. The surgical defects were made in the nasal bone of a rabbit and BIOPEX® was implanted in the left side and no material in the right side. Two-hall-absorbable plate and two screws (Super FIXSORB®-MX) were fixed across the defect in each side. The rabbits were sacrificed at 1, 4, 12 and 24 weeks postoperatively, and formalin-fixed specimens were embedded in acrylic resin. The specimens were stained with hematoxylin and eosin. For immune-histochemical analysis, the specimens were treated with bone morphogenetic protein-2 (BMP-2) antibodies. Finally, these were evaluated microscopically.

Results: New bone formation was observed in the region of absorbable plate and nasal membrane after more than 4 weeks. The area of BIOPEX® + new bone was significantly
larger than that of control side after 1, 4 and 12 weeks (P<0.05). The number of BMP-2 stained cell in experimental side was significantly larger than that in control side after 4 and 12 weeks (P<0.05).

**Conclusion:** This study suggested that the use of absorbable plate (Super FIXSORB®-MX) in combination with BIOPEX® were useful and both of Super FIXSORB®-MX and BIOPEX® could provide adequate bone regeneration.

Key word: Absorbable plate  
Self-setting α-tricalcium phosphate  
Bone morphogenetic protein  
Orthognathic surgery
**Introduction**

Late, absorbable plate systems are being used increasingly in oral and maxillofacial surgery.\(^1,2\) The biodegradable devices have major advantages over conventional metallic implants. There is no need for a second operation to remove the implant. There is less risk of weakening of the fixed bone because of stress shielding and there is no risk of metallic corrosion. However, several problems remain, including mechanical weakness,\(^3,4\) late foreign body reactions, osteolytic change, and micro-movement of fixed bone caused by a low initial stability.\(^5,6,7\)

Recently, resorbable bone fixation devices (Super-FIXSORB\(^\text{®}\)-MX. Takiron Co. Ltd, Osaka) have been developed for use in orthopedic or cranio-facial, oral and maxillofacial or plastic and reconstructive surgeries.\(^8,9,10\) These devices are made from composites of uncalcined and unsintered hydroxyapatite (u-HA) particles and poly-L-lactated (PLLA), and they are produced by a forging process, which is a unique compression molding, and machining treatment. They have a modulus of elasticity close to that of natural cortical bone, and they can retain a high strength during the period required for bone healing. They can also show optimal degradation and resorption behavior, osteoconductivity, and bone bonding capability.
On the other hand, Monma et al.\textsuperscript{11} have originally developed a self-setting cement-type calcium phosphate material consisting of $\alpha$-TCP, dicalcium phosphate dibasic (DCPD) and tetracalcium phosphate monoxide (TeCP). According to their extensive studies, this cement-type material could be refined, demonstrating better biocompatibility and direct integration to bone without any participation of peripheral soft tissue.\textsuperscript{12-16} As it is free of the infiltration with time of residual monomers of methacrylate resin, which has long been used for orthopedic treatment, this self-setting cement came to be rapidly highlighted for clinical use in Japan.

In orthognathic surgery, use of bone graft was sometimes needed in the contact area between segments in sagittal split ramus osteotomy or Le Fort I osteotomy.\textsuperscript{17,18} Use of a plate and screw system only was enough to fix the segments and maintain the stability, according to previous reports.\textsuperscript{1,2} However, the absorbable plate system was used in cases where the space between the bony segments was wide, and whether proper rigidity and stability can be achieved was also considered. Therefore, the combined use of segmental fixation with absorbable plate system and filling in the space between the segments were assumed to be clinically necessary.

The purpose of this study was to histologically and immuno-histochemically evaluate bone formation using both self-setting $\alpha$-tricalcium phosphate ($\alpha$-TCP; BIOPEX\textsuperscript{®}) and
absorbable plate (Super FIXSORB®-MX) in rabbit cranium bone.

**Materials and Methods**

The experimental protocol was approved by the Institutional Committee for Animal Care, Kanazawa University. Sixteen male Japanese white rabbits (12-16 weeks, 2.5-3.0 kg) were used in this study.

**Surgical procedure**

The entire procedure was performed under sterile conditions. First, the animals were anesthetized with sodium pentobarbital (25 mg/kg) by injection into the lateral ear vein. After the hair in the nasal region was shaved, 1.8 ml of 2% lidocaine containing 1:80,000 epinephrine was administered to the surgical site. Both the nasal bone and nasoincisinal suture line were exposed via a perpendicular incision. With the use of a fissure bur, four nasal bone windows were outlined. A surgical defect (3×20 mm) was made with a fissure bur using continuous saline irrigation. The interval between screw halls of the uHA/PLLA plate was approximately 4 mm, so that the size of more than 3 mm
could be chosen. The surgical defect was divided two areas; the right was the control side and the left was the experimental side (Fig 1).

*Implant materials and fixation plate*

The implant materials were $\alpha$-TCP/DCPD/TeCP cement (BIOPEX®, Mitsubishi Materials, Yokose, Chichibu, Japan). The cement powder consisting of $\alpha$-TCP(75% wt), DCPD (5% wt) and TeCP (20% wt) were mixed with an aqueous solution containing 12 % sodium succinate and 5 % sodium chondroitin sulfate to prepare the cement paste. A power/liquid ratio of 3.0 was chosen for ease of mixing and sufficient strength as a filling material, according to the results of Kurashina et al.\textsuperscript{12} The cement paste was implanted in the defect on the left side area (3×10 mm). No material was implanted into the right side. On each side, Super-FIXSORB®-MX plate (straight, 2 halls) and two screws (2×5 mm) were fixed across the surgical defect (Fig.1).

*Histological examination*

The rabbits were sacrificed at 1, 4, 12 and 24 weeks postoperatively and the specimens were collected for hematoxylin and eosin staining and also for immunno-histochemical analysis. After fixation with 10% phosphate-buffered formalin,
the specimens with the titanium mesh were dehydrated in ethanol and technovit 7200VCL (Kultzer and Co., GmbH, Wehreim, Germany) and then embedded in acrylic resin. The embedded blocks were trimmed by a cutter and ground by abrasive paper. Thereafter, the sections were further ground to a final thickness of about 10 μm. Finally, the specimens were stained with hematoxylin and eosin and examined under the microscope. The observation area was located between two screws and between absorbable plates and the nasal membrane (Fig.2). The bone or BIOPEX® area ratio was measured with image software (Scion image, Scion corporation ML, USA).

The prepared sections were deacrylated in 2-methoxyethyl acetate, inhibited by endogenous peroxidase with 0.3% hydrogen peroxide and blocked in 10% normal serum prior to staining. For immunostaining, commercially available monoclonal anti-BMP-2 antibodies (Dako North America, Inc, CA, USA) were used. Sections were incubated overnight with these primary antibodies at 4ºC in a humidified chamber. A biotinylated goat anti-mouse IgG antibody (Wako Junyaku Inc., Osaka, Japan) was used as the secondary antibody. The Vectastain-Elite ABC kit detection system, DAB revelation kit and DAB enhancing solution (Wako Junyaku Inc., Osaka, Japan) were used to complete the immunostaining. Finally, a light Meyer’s hematoxylin counter stain was applied. The sections were then dehydrated in alcohol and mounted for light
microscopy to count the number of positively stained active cells in the regeneration site. The observation area was located near the nasal membrane in the middle area between two screws (Fig. 2). The number of BMP-2 stained cells per voluntary 1000 cells in this area was counted by hand using high magnification photomicrograph (×100).

Statistical Analysis

Data of all the implanted materials were statistically analyzed with Stat View 4.5 (ABACUS Concepts, Inc., Berkeley, CA, USA). Differences between groups were analyzed by non-paired comparison using Scheffe’s F test. Time-dependent changes were examined by analysis of variance (ANOVA). Differences were considered significant at P< 0.05.

Results

Healing progressed uneventfully in all animals and no postoperative complications were noted during the 24-week observation period. After resting for 3-6 days postoperatively, the animals could move and leap without any notable pain or limitation.
Absorbable plate region

After 1 week, on the experimental side, fibrous tissue or cell component including osteoblast was not found between absorbable plate and BIOPEX®, because there was no space between the plate and BIOPEX® paste. On the control side, fibrous tissue or cell components were not observed and the surgical defect area was empty (Figs.3 and 4).

After 4 weeks, fibrous tissue or cell components including osteoblasts were recognized on both the experimental and control sides. In the experimental group, fibrous tissue was located in the gap between the absorbable plate and BIOPEX®. However, resorption of BIOPEX® by osteoclasts was not seen in the experimental side. Absorbable plate resorption was not seen in both sides (Fig. 5).

After 12 weeks, fibrous tissue decreased and new bone formation was found in both sides. On the experimental side, resorption of surrounding BIOPEX® derived from osteoclasts was observed. Although absorbable plate resorption was not seen, new bone formation was observed under the plate in both sides (Fig. 6).

After 24 weeks, in the control side, new bone formation increased gradually. However, similar findings were seen up to 12 weeks in the experimental side (Fig. 7).
Nasal membrane region

After 1 week, the nasal membrane was still not significantly regenerated in both sides. However, after 4, 12 and 24 weeks, the nasal membrane repaired in both sides and similar findings were observed in both sides. After 12 weeks, resorption of BIOPEX® in nasal membrane region showed a tendency to be less than that in the absorbable plate region (Figs.3-7).

Bone area

With regard to the bone area, the subjects were divided into 4 groups as follows and statistical calculations performed.

Area A: Bone area in the experimental side,

Area B: Bone area in the control side,

Area C: BIOPEX area in experimental side

Area D (Area A+C): Bone area + BIOPEX® area.
The time-course change was significant, however there was not significant difference between the groups by repeated measure ANOVA (between subjects; F=373.634, df=3, P=0.0048; within subjects; F=69.152, df=9, P=0.4997). Although there was no significant difference between area A and B in all the periods, bone area in both groups increased gradually. In contrast, area C decreased gradually. Although area C was significantly larger than area B after 1 week (P=0.082), there was no significant difference between area C and B after 4, 12 and 24 weeks. Area D was significantly larger than area A after 1 (P=0.0009), 4 (P=0.0030) and 12 weeks (P=0.0375), however, there was no significant differences between areas D and A after 24 weeks. Area D was significantly larger than area B after 1 (P=0.0003), 4 (P=0.008) and 12 weeks (P=0.0156), however, there was no significant differences between areas D and B after 24 weeks. Area D was significantly larger than area C after 12 weeks (P=0.0482), however, there was no significant differences between areas D and C after 1, 4 and 24 weeks (Fig. 8).

*Number of BMP-2 stained cells*

Fibrous tissue and cell components including osteoblasts did not exist in the
observation region so that the number of BMP-2 stained cells could not be counted after 1 week. The time-course change was not significant, and there was no significant difference between the groups by repeated measure ANOVA. However, the number of BMP-2 stained cells in the experimental side was significantly larger than that in the control side after 4 weeks (P=0.0002) and 12 weeks (P=0.0254) (Fig. 9).

Discussion

Orthognathic surgery is frequently performed all over the world, and Le Fort I osteotomy and sagittal split ramus osteotomy are well-known standard surgical procedures.

In Le Fort I osteotomy, incomplete ossification of osteosynthesis is one of the major problems. If the size of the defect between the advanced inferior maxillary segment and the superior segment exceeds 3mm at the levels of the piriform rim and zygomaticomaxillary junction following Le Fort I osteotomy, use of a bone graft may be required for stabilization. Furthermore, bone recovery would be inadequate if there is a defect of more than 3 mm between the segments along the line of osteosynthesis. The line of osteosynthesis would involve a fibrous tissue rather than osseous tissue. In this
case, resistance to relapse would be via this fibrous tissue and the initial plate and screw rather than a robust osseous tissue.\textsuperscript{17,18} However, absorbable plate systems are being used increasingly in oral and maxillofacial surgeries. In our previous report, there was no difference in the stability following Le Fort I osteotomy without bone graft between a titanium plate group and an absorbable plate group in most frontal and lateral cephalometric measurements. However, a significant difference in the A point in the lateral cephalometric measurements was found between the two groups.\textsuperscript{1} This suggested that the difference between titanium and the absorbable plate could affect the stability following Le Fort I osteotomy.

Our previous study showed that the gap between the proximal and distal segments could fill up with new bone after SSRO with titanium or absorbable plates, even if there were few bony contacts between the segments. However, a concave outline was observed in some cases in the study, although the square of ramus increased significantly.\textsuperscript{19} If healing of the periosteal membrane at the incision area is complete, it can prevent invasion of mucosal endothelial cells into the gap between segments.

Ideally, when the absorbable plate is used in Le Fort I osteotomy or SSRO, significant strength by bony healing between segments is necessary to maintain skeletal stability after a decrease in strength in the absorbable plate and screw.
uHA/PLLA plate system (Super-FIXSORB®-MX) which have completed clinical
tests in orthopedic, oral and maxillofacial surgeries exhibit total resorbability and
osteological bioactivity such as the ability to directly bond to bone and
osteoc conductivity\textsuperscript{20} as well as good biocompatibility and high stiffness retainable for a
long period of time to achieve bone union.\textsuperscript{21} Shikinami et al. documented the complete
process of bioresorption and bone replacement of rods made of forged composites of
unsintered hydroxyapatite particles/poly L-lactide (F-u-HA/PLLA) implanted in the
femoral medullary cavities of rabbits.\textsuperscript{10} From the results, it was found that
morphological changes during biodegradation and bone replacement in the proximal
medullary cavity up to 4.5 years, and molecular weight and the bending strength had
decreased to 50 KDa and 200 MPa after 6 months. Therefore, if the strength of the
absorbable plate decreases and the bony healing between segments is not complete at
least 6 months after osteotomy, the skeletal stability can not sustain for a long time.

To achieve osseous healing, a grafted material needs to serve as a scaffold for
the migration, attachment, and subsequent differentiation of osteoprogenitor cells from
the recipient bed.\textsuperscript{22,23} Various types of biocompatible graft materials have been
developed in consideration of bone regeneration and augmentation as well as further
procedures such as dental implantation\textsuperscript{24-26}. Autogenous bone graft has drawbacks
such as the requirement of a second operation, which is sometimes accompanied by
broad invasion, and a restricted source of materials. Alloplastic materials composed of
inorganic substances such as tricalcium phosphate (TCP) or hydroxyapatite show
biocompatibility and osteoconductivity, a major reasons why this type of material
has been widely employed for hard tissue repair. α-TCP is predominant during bone
resorption in the early stage of bone healing; this may lead to technically difficulties
in both the filling of particles to fit the bone defects and their delivery to form the
desired shape. However, cement type calcium phosphate material in paste form
processes self-setting property in situ, thereby solving these technical problems. The
characteristic properties of the self setting materials work to prevent intervention for
undesirable connective tissues or dispersion of the calcium phosphate particles in to the
surrounding tissue.

Bone morphogenetic proteins (BMPs) are active bone-inducing factors that
act on immature mesenchymal cells, including osteoblasts, resulting in osteogenesis.
So far, several types of BMPs have been isolated using molecular cloning, and
recombinant BMP molecules have been synthesized. Recombinant human BMP-2
(rhBMP-2) is a molecule that powerfully induces bone generation in vivo. Bone
formation depends mainly on the number of osteoblastic cells rather than the activity of
the osteoblasts.\textsuperscript{47} The recruitment of osteoblastic cells plays a crucial role in osteogenesis. Within subperiosteal implantation sites, BMP induces new bone formation in heterotrophic, intramuscular and orthotropic sites \textit{in vivo}.\textsuperscript{48} It is thought that BMP induces the differentiation of pre-vascular mesenchymal connective tissue cells into bone and cartilage. Therefore, an immuno-histochemical study using BMP antibody was very adequate to examine the possibility of bone-induced effects, but there have been no reports on the expression of BMP induced by $\alpha$-TCP. In our previous study of onlay graft using rabbit mandible,\textsuperscript{49} in the immuno-histochemical examination after treatment with BMP-2 antibody, when the autologous group was compared with the control and $\beta$-TCP groups, the cell numbers increased significantly, and there was no significant difference between control and $\beta$-TCP in terms of cell number for 2, 4 and 8 weeks. This suggested that bone inductive activity of $\beta$-TCP could be low. On the other hand, Yuan et al.\textsuperscript{50} founded that porous $\beta$-TCP ceramic could induce bone formation in soft tissue in dogs, but porous $\alpha$-TCP ceramic could not. They concluded that no bone formation in $\alpha$-TCP might have resulted from its higher resorbability or more accurately, due to its rapid dissolution. The rapid dissolution of $\alpha$-TCP also affected its cell-mediated resorption. Self-setting of $\alpha$-TCP (BIOPEX\textsuperscript{®}) in this study was different from that of porous $\alpha$-TCP so that they cannot be readily compared.
In this study, fibrous tissue and cell components including osteoblasts were absent in the observation region, therefore, the number of BMP-2 stained cells could not counted after 1 week. This suggested that tight contact could be obtained between the absorbable plate and BIOPEX®. The number of BMP-2 stained cells in the experimental side was significantly larger than that in the control side after 4 and 12 weeks. This suggested that osteoblast cells were localized onto the BIOPEX® and the absorbable plate. The deposited bone matrices on these materials implied a coupling between osteoclasts and osteoblasts. Resorption of the BIOPEX® was observed after 12 weeks at the region of the absorbable plate, but that was after 24 weeks at the region of the nasal membrane. This suggested that the resorption of the BIOPEX® was comparatively slow, but different in the region of the implant. In the study of Hao et al. using rabbit femoral cortical bone, a range of osteoclasts accumulated on the surface of the BIOPEX®, whilst many osteoblasts were localized on the surface opposing the BIOPEX® from days 5 to 10. However, remnants of the BIOPEX® particles were present in the new born with a profile of compact bone on days 30 and 40. The BIOPEX® was so hard that the osteoclasts could not resorb rapidly.

Although, the number of BMP-2 stained cells in the experimental side was significantly larger than that in the control side, there was no difference in the new bone
formation area between the control and experimental sides. For the reason, it was suggested that area of the BIOPEX® occupied a large area of the surgical defects and thus the bone formation area became narrow. However, the total area of the new bone and BIOPEX® was constantly larger than the new bone of the control sides until 12 weeks. This suggested that strength in the space of the surgical defects could be supported by not only the absorbable plate, but also implant of BIOPEX® and sequential new bone formation. However, after 24 weeks, there were no significant difference in the bone area among all areas. This might be because new bone formation increased in the control side but the area of BIOPEX® decreased due to resorption in the experimental side after 24 weeks.

On the other hand, although resorption of absorbable plates was not observed at all within 24 weeks, osteoblasts were observed under the plate after 4 weeks. In other words, this study also proved that the Super FIXSORB®-MX could induce new bone formation, as previously reported.

In conclusion, this study suggested that the use of an absorbable plate (Super FIXSORB®-MX) in combination with BIOPEX® was useful and both Super FIXSORB®-MX and BIOPEX® could provide adequate bone regeneration and maintain strength and stability at the surgical bone space.
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Legends:

**FIGURE 1.** Intra-operative findings.

(A) Bone defect was made at the nasal region.

(B) Surgically-created bone defects. (a) Right side, Control side: no material was implanted within the defects. (b) Left side, experimental side: BIOPEX® was implanted within the defects.

(C) In each side, Super-FIXSORB®-MX plate (straight, 2 halls) and two screws (2×5 mm) were fixed across the surgical defect.

**FIGURE 2.** The coronal section of the nasal region with implanted materials. *: The blue colored area between the screws and under the absorbable plate indicates the area to measure new bone and BIOPEX®. **: This shows the area to measure BMP-2 stained cells. A; absorbable plate (Super-FIXSORB®-MX), N; nasal membrane, NB; new bone, M; mother bone, T; BIOPEX®

**FIGURE 3.** Photomicrographs of the control side. (A) 1 week, (B) 4 weeks, (C) 12 weeks (D) 24 weeks (HE staining, original magnification ×40), P: positive stained
cell. A; absorbable plate (Super-FIXSORB®-MX), N; nasal membrane, NB; new bone, M; mother bone, F; fibrous tissue.

FIGURE 4. Photomicrographs of the experimental side after 1 week. (A) HE staining, original magnification ×20, (B) absorbable plate region, HE staining, original magnification ×400, (C) nasal membrane region, HE staining, original magnification ×400. A; absorbable plate (Super-FIXSORB®-MX), N; nasal membrane, M; mother bone, T; BIOPEX®. Fibrous tissue including cell component was not found between absorbable plate and BIOPEX®.

FIGURE 5. Photomicrographs of the experimental side after 4 week. (A) HE staining, original magnification ×20, (B) absorbable plate region, HE staining, original magnification ×400. The gap was observed between the absorbable plate and BIOPEX®. (C) nasal membrane region, HE staining, original magnification ×400. (D) nasal membrane region, BMP-2 antibody staining, original magnification ×400. A; absorbable plate (Super-FIXSORB®-MX), N; nasal membrane, M; mother bone, T; BIOPEX®, S; BMP-2 stained cell.
FIGURE 6. Photomicrographs of the experimental side after 12 week. (A) HE staining, original magnification × 20, (B) absorbable plate region, HE staining, original magnification × 400, The gap was observed between the absorbable plate and BIOPEX® (C) nasal membrane region, HE staining, original magnification × 400. (D) nasal membrane region, BMP-2 antibody staining, original magnification × 400. A; absorbable plate (Super-FIXSORB®-MX), N; nasal membrane, M; mother bone, T; BIOPEX®, S; BMP-2 stained cell.

FIGURE 7. Photomicrographs of the experimental side after 24 weeks. (A) HE staining, original magnification × 20, (B) absorbable plate region, HE staining, original magnification × 400, New bone formation was observed in the gap between the absorbable plate and BIOPEX® (C) nasal membrane region, HE staining, original magnification × 400. (D) nasal membrane region, BMP-2 antibody staining, original magnification × 400. A; absorbable plate (Super-FIXSORB®-MX), N; nasal membrane, M; mother bone, T; BIOPEX®, S; BMP-2 stained cell.

FIGURE 8. The ratio of bone and BIOPEX®. Area A; Bone area in the experimental side, Area B; Bone area in the control side, Area C; BIOPEX area in the
experimental side, Area D (Area A+C); Bone area + BIOPEX® area. * shows significant difference at P<0.05.

**FIGURE 9.** Number of BMP-2 positive cells. * shows significant difference at P<0.05.
Fig. 5
Fig. 7
Fig. 8
Fig. 9

No. of positive cells (week)

(%)