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Bone formation with alloplastic graft substitutes in critical-sized rat calvarial defects

조선대학교 대학원

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노 기 표

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백서 두개부 결손부의 이식재 성분에 따른 골 형성

2010년 8월 25일

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지도교수 김 수 관

이 논문을 치의학 박사학위신청 논문으로 제출함

2010년 4월

조선대학교 대학원

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조선대학교 대학원

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국문초록

백서 두개부 결손부의 이식재에 따른 골 형성

노 기 표
지도교수 : 김 수 관
조선대학교 치의학과 구강악안면외과학 전공

본 연구의 목적은 HA와 β-TCP 성분의 비율에 따른 골형성 정도에 대한 차이가 있는지를 평가하는 데 있다.

60마리의 쥐 두개부에 8mm 직경의 critical-size의 골결손부를 형성하고 HA/β-TCP의 조성이 다른 골이식재 4가지: (1) unfilled defect, (2) HA (100%), (3) HA(70%)/β-TCP(30%), (4) HA(30%)/β-TCP(70%), (5) β-TCP(100%)를 사용하여 골이식을 시행한 후 4주후, 8주후에 희생시켜 광학현미경으로 골형성 면적을 비교 평가하여 다음과 같은 결과를 얻었다.

1. 수술 후 4주군에서 β-TCP(100%)를 이식한 경우에서 0.75±0.21mm^{2}로 약간 더 많은 골형성 면적을 보였으나 다른 그룹과 비교시 통계적 유의성이 없었으며 나머지 그룹간에도 유의할 만한 차이는 없었다.
2. 수술 후 8주군에서는 HA (100%)와 HA(30%)/β-TCP(70%)를 이식한 경우에서 각각 2.60±1.03mm^2, 2.56±0.93mm^{2}의 골형성이 관찰되어 나머지 3그룹보다 많은 골형성을 보였으며 이는 통계적으로 유의성이 있었다.
3. HA (100%)와 HA(30%)/β-TCP(70%)의 2 그룹간의 차이는 유의성이 없었으며 control, HA(70%)/β-TCP(30%), β-TCP(100%)의 3 그룹간의 차이도 유의성이 없었다.

위와 같은 결과를 바탕으로 보았을 때 HA 100%와 HA(30%)/β-TCP(70%)를 사용하여 골이식을 시행할 경우 형성되는 신생골의 양을 증가시켜 양호한 결과를 얻을 수 있을 것으로 예상된다.

Introduction

Several studies have revealed that bone grafts and other regeneration surgeries are useful in repairing bone defects, and diverse methods have been applied. Bone graft materials are used for reconstructive surgery include autogenous bones, allogenic bones, and alloplastic bones. Among these, alloplastic bones are the most readily obtained, and they obviate the need for performing surgery on another area to harvest bones, thus reducing complications, decreasing costs, and facilitating manipulation to individual needs. In addition, alloplastic bones have the advantage of minimizing the risk of autoimmune diseases or infection. However, in comparison with autogenous bone, the regeneration activity of alloplastic bone is low, and thus combining alloplastic with autogenous bone is recommended.[1-7] Among alloplastic bone materials, those that have attracted the most attention are hydroxyapatite (HA) and beta-tricalcium phosphate (β-TCP).

HA/β-TCP provides 90% multiporosity and an internal environment that cells can invade. Consequently, it can play the role of a fine cell carrier and facilitate the improvement of bone formation by undifferentiated mesenchymal cells. It has been reported that with proper proportions of HA and β-TCP, calcium and phosphate ions are released from the grafted area, resulting in the acceleration of new bone formation.[8,9]

The purpose of this study was to evaluate the level of bone formation is different depending on the ratio of these two components.

Materials and methods

Materials

Sixty Wistar rats, weighing 250-300g, were used, and prior to the initiation of experiments, the rats were allowed to adapt to their new environment for 7 days. The rats were divided to five groups according to bone graft materials, and again divided into two groups according to the sacrifice time (4 weeks or 8 weeks post-surgery). The five graft material groups were (1) unfilled defect (control), (2) HA (100%) grafts (Calcitite HA 2040 Particles®, Zimmer dental Inc, USA), (3) HA (70%)/β-TCP (30%) grafts (Osteon®, Genos Co., Korea), (4) HA (30%)/β-TCP (70%) grafts (Bio-C®, Cowellmed Co,, Korea), and (5) β-TCP (100%) grafts (Synthograft® Bicon Inc, USA). The particle size of all graft materials was 0.51.0 mm, except that Calcitite HA 2040 Particles® had a particle size of 1-2 mm.

Methods

For anesthesia, 10 mg/kg (body weight) zolazepam solution (Zoletil ® 50, Virbac, So Paulo, Brazil) and 0.3 mg/kg Rompun® (Bayer Korea, Co.) were injected intramuscularly. After the induction of sufficient anesthesia, the cranial area was shaved and the skin exposed, and 2% lidocaine was injected into the operating site. The area was sterilized using Potadine, an incision approximately 25 mm long was made, subcutaneous tissues were dissected, and the bone tissue in the cranial area was exposed by lifting the periosteum bilaterally. To prevent adjacent tissues from interfering the surgery, under the lifted condition, using a low-speed carbide bur, a cranial defect area 8 mm in diameter was formed. Bone removal was performed under sufficient irrigation, and thus the injury of adjacent tissues was

minimized. The defect size was measured by a caliper.

A low-speed rotation engine (SSWHITE®, USA) was used at 1,500 rpm, and the bur used in the rotation engine was a carbide bur 2 mm in diameter. In the cranial area, a defect in the critical size was formed, bone graft was performed with each graft material, simple suture was performed with 4-0 polyglactin (Vicryl®, Johnson & Johnson), and Gentamycin was injected to prevent infection.

Preparation of tissue samples and examination

At 4 weeks and 8 weeks after surgery, animals of each group were anesthetized and sacrificed, and the cranial bone defect area and adjacent healthy bone tissues were resected en bloc. The harvested tissues were fixed in 10% neutral formalin solution for 24 hours, decalcified with nitric acid (De-Cal Rapid, Pational Diagnosis, Atlanta, USA) for 24 hours, and embedded in paraffin according to a conventional method. The samples were sectioned at 5㎛ in thickness, stained with hematoxylin and eosin, and examined by light microscopy.

Statistical analysis

The bone formation rate according to the bone graft material and the post-surgical follow up intervals were compared by T-test, ANOVA, and Tukey HSD. Statistical difference was considered significant if $p < 0.05$.

Results (Fig. 1 – 10)

4 weeks after grafting

In the four groups excluding the control group, grafted materials were observed together with newly formed bone tissues. The control group exhibited a lower volume of new bone formation than the experimental groups. In the group grafted with 100% β-TCP, slightly more bone formation was observed, but it was not statistically significant.

8 weeks after grafting

Although large differences were not detected in the 4-week groups, at 8 weeks, in the group grafted with 100% HA (Calcitite HA 2040 Particles®) or with 30% HA/70% β-TCP, a statistically significant increase in bone formation was observed compared with the other three groups.

The level of bone formation from each combination of graft material and elapsed time was calculated as cross-sectional area (mm^2). When the results were compared, there were no significant differences among the 4-week groups, while in the 8-week results, significantly more bone formation was observed in the group grafted with 100% HA and in the group grafted with 30% HA/70% β-TCP compared with the other three 8-week groups. Among the remaining three groups (control, 70% HA/30% β-TCP, and 100% β-TCP), no statistically significant variations were detected (Table I).

Table I. New bone formation area (mm^2) from each grafted material and time point

Groups	4 weeks	8 weeks
Control	0.39±0.17	0.56±0.14
HA (100)	0.53±0.31	2.60±1.03*,**
HA:β-TCP (70:30)	0.53±0.26	0.56±0.29*
HA:β-TCP (30:70)	0.61±0.12	2.56±0.93*,***
β-TCP (100)	0.53±0.23	0.75±0.21*

*Statistically more significant new bone formation at experimental groups at 8 weeks than control at 8 weeks.

**Statistically more significant new bone formation at HA group at 8 weeks than HA:β-TCP(70:30) and β-TCP at 8 weeks.

***Statistically more significant new bone formation at HA:β-TCP (30:70) at 8 weeks than HA:β-TCP (70:30) and β-TCP at 8 weeks.

Discussion

The composition of HA and β-TCP is determined by the combination of the stability of HA and the solubility of β-TCP. The materials are grafted in the body and degraded slowly, and as bio-materials, they release calcium and phosphate ions, thus accelerating the formation of new bone. The ratio of HA to β-TCP is determined by the composition of calcium and when this ratio is low (HA<β-TCP), the synergistic effect becomes high. In addition, the size of graft particles, the levels of macro-porosity and micro-porosity, sintering temperature are also factors that influence the bone formation. The advantage of HA and β-TCP bone graft materials is that they fuse strongly with host bones in comparison with other graft materials.[10,11]

Artzi et al.[12] used a mixture of HA/β-TCP and autogenous bone at a ratio of 1:1 as graft materials and performed maxillary sinus lifts on 28 patients. The grafts were examined histologically after 6 and 9 months, and they found that the bone formation rate increased with time. In a study by Zijderveld et al.[13] that examined the change in height during 45 years after maxillary sinus lift, the group grafted only with autogenous bones was compared with the group grafted only with 100% β-TCP. The vertical height was decreased, but there was no significant difference between the two groups. In addition, in a study comparing bovine hydroxyapatite with non-ceramic resorbable hydroxyapatite, after 12 months, bovine hydroxyapatite showed a higher bone formation rate.[14]

Similar to this study, in the report of Park et al.[15] in which a critical-size defect was formed in the rat cranial area and bone grafts with various graft materials were performed, the group grafted only

with new hydroxyapatite (N-HA) isolated from eggshells, the group grafted with a mixture of N-HA and calcium sulfate (CS), and the group grafted with bovine bone (Bio-Oss®) were compared after 6 weeks and 12 weeks. They found that N-HA grafts showed a high rate of new bone formation similar to that of the rats receiving N-HA mixed with CS. In addition, some have reported that when β-TCP is grafted together with bone marrow to the cranial area, the bone formation rate increases, and in some areas where β-TCP is present, it is replaced with bone.[16] In investigations of the particle size of graft materials, a size of 0.3~0.5 mm has produced the best results.[17]

Lange et al.[18] cultured inflammatory cells with HA and β-TCP and found that pure β-TCP produced proinflammatory cytokines similar to HA but in smaller quantities. In addition, these two materials interfere with the action of osteoblasts by enhancing PGE_2 synthesis.[19,20]

Using autogenous bones and HA/β-TCP, Zafiropoulos et al.[21] examined the regeneration process of the deep intrabony pocket and evaluated the vertical bone gain and relative bone gain. They reported that in the cases that used HA and β-TCP together, significantly superior clinical outcomes were obtained compared to the group that used only autogenous bones. In this study, the 100% HA group and the 30% HA/70% β-TCP group exhibited significantly better bone formation than groups that received 70% HA or 100% β-TCP.

Conclusion

In the rat cranial area, a critical-size bone defect 8 mm in diameter was generated, and bone grafts were performed with four types of bone graft materials: (1) HA (100%), (2) HA (70%)/β-TCP (30%), (3) HA (30%)/β-TCP (70%), and (4) β-TCP (100%). A fifth group of negative controls did not receive any graft. The animals were sacrificed 4 weeks or 8 weeks post-surgery, and the bone formation cross-sectional area (mm^2) was compared and evaluated by light microscopy.

1. In the 4 weeks after surgery and grafted with β-TCP (100%), bone formation was 0.75 ± 0.21 mm^2, which was slightly greater than in the other groups, but this was not statistically significant ($P>0.05$).
2. In the 8 weeks after surgery, grafted with HA (100%) or with HA (30%)/β-TCP (70%) had 2.60 ± 1.03 mm^2 and 2.56 ± 0.93 mm^2 bone formation, respectively. These rates of bone formation were significantly higher than the other three groups ($P<0.05$).
3. In the 8 weeks after surgery, among the remaining three groups (control, 70% HA/30% β-TCP, and 100% β-TCP), no statistically significant variations were detected ($P>0.05$).

Based on the above results, it is anticipated that grafts performed with 100% HA or 30% HA/70% β-TCP will yield favorable results in terms of the formation of new bone. More studies will be required to confirm these results.

References

1. Kim YK, Kim SG, Byeon JH, Lee HJ, Um IU, Lim SC, Kim SY. Development of a novel bone grafting material using autogenous teeth. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2010;109:496-503.
2. Kim YK, Yun PY, Kim SG, Kim BS, Ong JL. Evaluation of sinus bone resorption and marginal bone loss after sinus bone grafting and implant placement. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2009;107:e21-8.
3. Hassan KS, Kassim A, Al Ogaly AU. A comparative evaluation of immediate dental implant with autogenous versus synthetic guided bone regeneration. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2008;106:e8-e15.
4. Kim SG, Park JS, Lim SC. Placement of implant after bone graft using J block allograft. Implant Dent 2010;19:21-8.
5. Moghadam HG. Vertical and horizontal bone augmentation with the intraoral autogenous J-graft. Implant Dent 2009;18:230-8.
6. Jensen SS, Bornstein MM, Dard M, Bosshardt DD, Buser D. Comparative study of biphasic calcium phosphates with different HA/TCP ratios in mandibular bone defects. A long-term histomorphometric study in minipigs. J Biomed Mater Res B Appl Biomater 2009;90:171-81.
7. Aghaloo T, Cowan CM, Zhang X, Freymiller E, Soo C, Wu B, Ting K, Zhang Z. The effect of NELL1 and bone morphogenetic protein-2 on calvarial bone regeneration. J Oral Maxillofac Surg 2010;68:300-8.
8. Bouler JM, LeGeros RZ, Daculsi G. Biphasic calcium phosphates: influence of three synthesis parameters on the HA/beta-TCP

ratio. J Biomed Mater Res 2000;51:680-4.
9. Kim YK, Yun PY, Lim SC, Kim SG, Lee HJ, Ong JL. Clinical evaluations of OSTEON as a new alloplastic material in sinus bone grafting and its effect on bone healing. J Biomed Mater Res B Appl Biomater 2008;86:270-7.
10. Appleford MR, Oh S, Oh N, Ong JL. In vivo study on hydroxyapatite scaffolds with trabecular architecture for bone repair. J Biomed Mater Res A 2009;89:1019-27.
11. Daculsi G, Laboux O, Malard O, Weiss P. Current state of the art of biphasic calcium phosphate bioceramics. J Mater Sci Mater Med 2003;14:195-200.
12. Artzi Z, Weinreb M, Carmeli G, Lev-Dor R, Dard M, Nemcovsky CE. Histomorphometric assessment of bone formation in sinus augmentation utilizing a combination of autogenous and hydroxyapatite/biphasic tricalcium phosphate graft materials: at 6 and 9 months in humans. Clin Oral Implants Res 2008;19:686-92.
13. Zijderveld SA, Schulten EA, Aartman IH, ten Bruggenkate CM. Long-term changes in graft height after maxillary sinus floor elevation with different grafting materials: radiographic evaluation with a minimum follow-up of 4.5 years. Clin Oral Implants Res 2009;20:691-700.
14. Artzi Z, Nemcovsky CE, Tal H, Dayan D. Histopathological morphometric evaluation of 2 different hydroxyapatite-bone derivatives in sinus augmentation procedures: a comparative study in humans. J Periodontol 2001;72:911-20.
15. Park JW, Jang JH, Bae SR, An CH, Suh JY. Bone formation with various bone graft substitutes in critical-sized rat calvarial defect. Clin Oral Implants Res 2009;20:372-8.
16. Shirasu N, Ueno T, Hirata Y, Hirata A, Kagawa T, Kanou M, Sawaki M, Wakimoto M, Ota A, Imura H, Matsumura T, Yamada

T, Yamachika E, Sano K. Bone formation in a rat calvarial defect model after transplanting autogenous bone marrow with beta-tricalcium phosphate. Acta Histochem 2009;27:1-8.
17. Zaner DJ, Yukna RA. Particle size of periodontal bone grafting materials. J Periodontol 1984;55:406-9.
18. Lange T, Schilling AF, Peters F, Haag F, Morlock MM, Rueger JM, Amling M. Proinflammatory and osteoclastogenic effects of beta-tricalciumphosphate and hydroxyapatite particles on human mononuclear cells in vitro. Biomaterials 2009;30:5312-8.
19. Sun JS, Tsuang YH, Liao CJ, Liu HC, Hang YS, Lin FH. The effects of calcium phosphate particles on the growth of osteoblasts. J Biomed Mater Res 1997;37:324-34.
20. Sun JS, Tsuang YH, Liao CJ, Liu HC, Hang YS, Lin FH. The effect of sintered beta-dicalcium pyrophosphate particle size on newborn Wistar rat osteoblasts. Artif Organs 1999;23:331-8.
21. Zafiropoulos GG, Hoffmann O, Kasaj A, Willershausen B, Weiss O, Van Dyke TE. Treatment of intrabony defects using guided tissue regeneration and autogenous spongiosa alone or combined with hydroxyapatite/beta-tricalcium phosphate bone substitute or bovine-derived xenograft. J Periodontol 2007;78:2216-25.

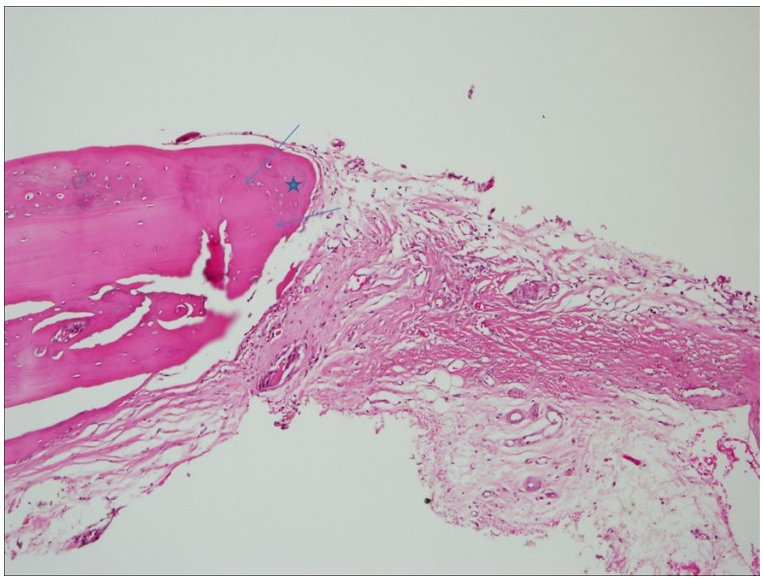

Fig. 1. Photomicrograph of control bone 4 weeks after surgery. Higher magnification demonstrated limited new bone formation (asterisk) around the defect margin (arrows) (H&E stain, ×100).

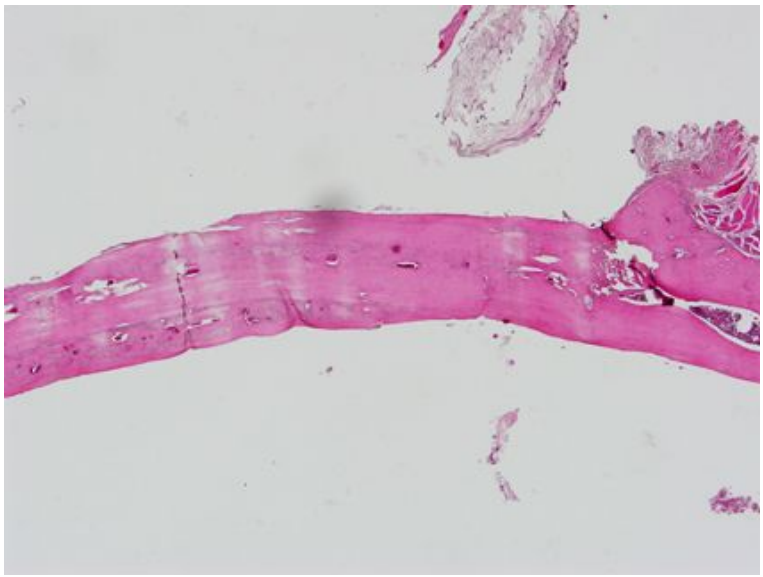

Fig. 2. Photomicrograph of control bone 8 weeks after surgery. The defect area was filled with continuous woven bone (H&E stain, ×40).

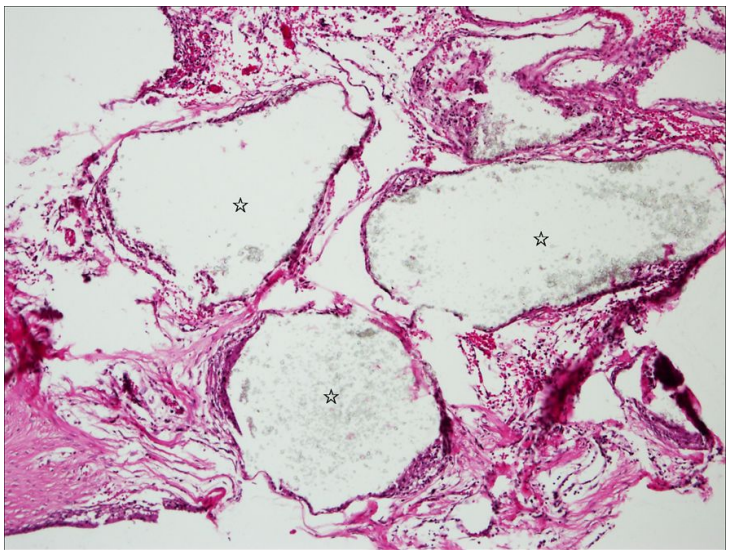

Fig. 3. Photomicrograph of HA (100%) graft 4 weeks after surgery. Higher magnification demonstrated no new bone formation around the implant chips (open asterisks) (H&E stain, ×100).

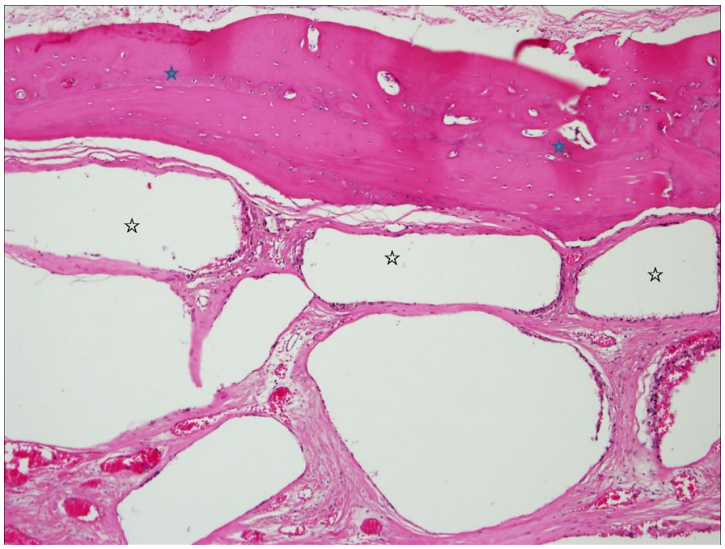

Fig. 4. Photomicrograph of HA (100%) graft 8 weeks after surgery. Higher magnification demonstrated continuous new bone formation (asterisks) along the implant chips (open asterisks) (H&E stain, ×100).

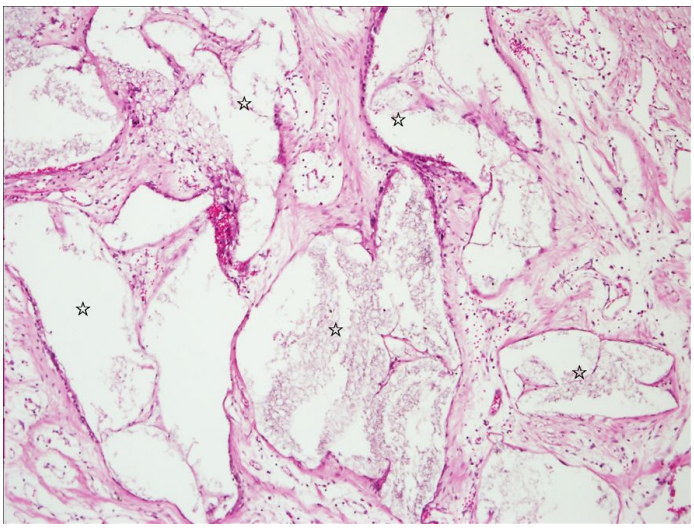

Fig. 5. Photomicrograph of HA (70%)/β-TCP (30%) graft 4 weeks after surgery. There was no discernible new bone formation in the defect area around the implant chips (open asterisks) (H&E stain, ×100).

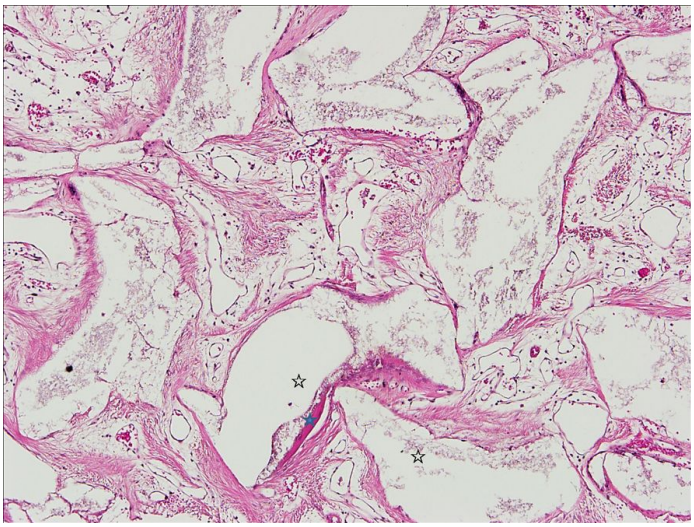

Fig. 6. Photomicrograph of HA (70%)/β-TCP (30%) graft 8 weeks after surgery. Higher magnification demonstrated some new-bone formation (asterisk) around the implant chips (open asterisks) (H&E stain, ×100).

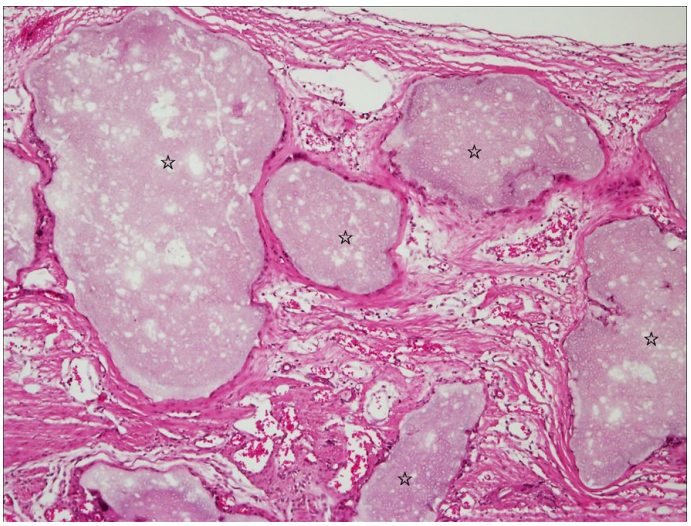

Fig. 7. Photomicrograph of HA (30%)/β-TCP (70%) graft 4 weeks after surgery. Higher magnification demonstrated no new bone formation around the implant chips (open asterisks) (H&E stain, ×100).

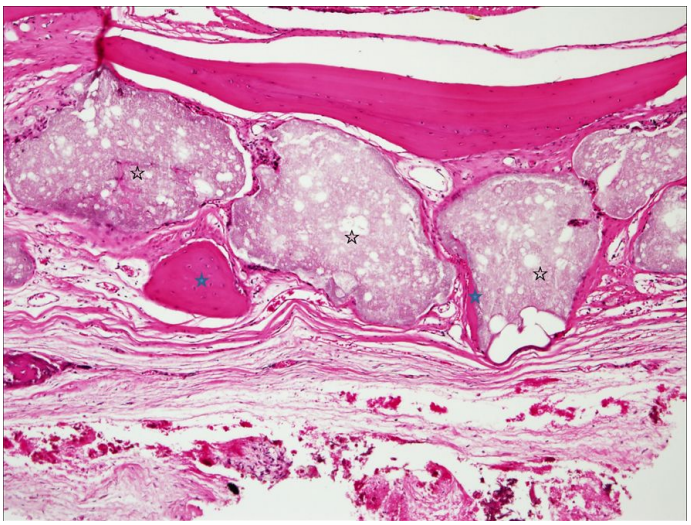

Fig. 8. Photomicrograph of HA (30%)/β-TCP (70%) graft 8 weeks after surgery. Higher magnification demonstrated some new bone formation (asterisks) around the implant chips (open asterisks) (H&E stain, ×100).

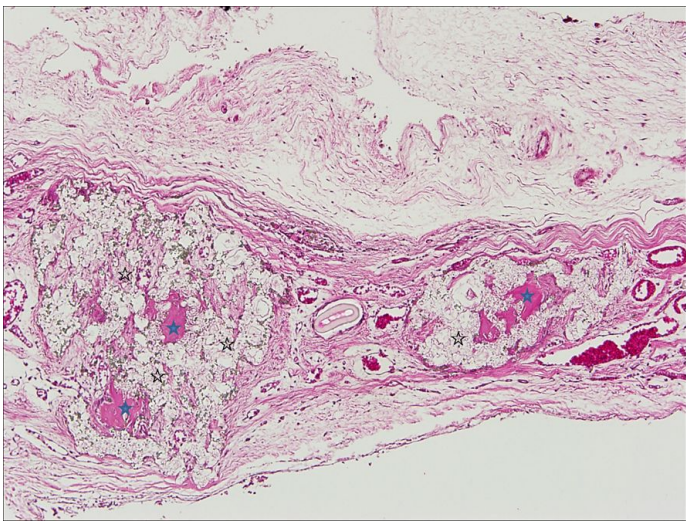

Fig. 9. Photomicrograph of β-TCP (100%) graft 4 weeks after surgery. Higher magnification demonstrated some new bone formation (asterisks) around the implant chips (open asterisks) (H&E stain, ×100).

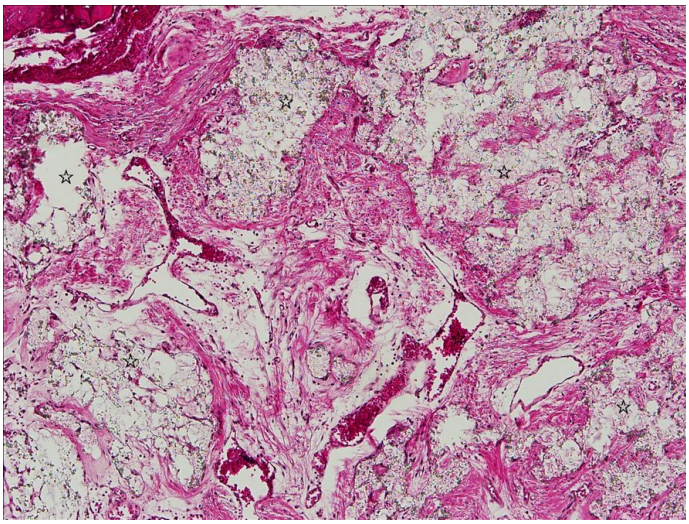

Fig. 10. Photomicrograph of β-TCP (100%) graft 8 weeks after surgery. There was no discernible new bone formation in the defect area around the implant chips (open asterisks) (H&E stain, ×100).

저작물 이용 허락서

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	영어 : Bone formation with alloplastic graft substitutes in critical-sized rat calvarial defects				

본인이 저작한 위의 저작물에 대하여 다음과 같은 조건아래 조선대학교가 저작물을 이용할 수 있도록 허락하고 동의합니다.

- 다 음 -

1. 저작물의 DB구축 및 인터넷을 포함한 정보통신망에의 공개를 위한 저작물의 복제, 기억장치에의 저장, 전송 등을 허락함
2. 위의 목적을 위하여 필요한 범위 내에서의 편집·형식상의 변경을 허락함. 다만, 저작물의 내용변경은 금지함.
3. 배포·전송된 저작물의 영리적 목적을 위한 복제, 저장, 전송 등은 금지함.
4. 저작물에 대한 이용기간은 5년으로 하고, 기간종료 3개월 이내에 별도의 의사 표시가 없을 경우에는 저작물의 이용기간을 계속 연장함.
5. 해당 저작물의 저작권을 타인에게 양도하거나 또는 출판을 허락을 하였을 경우에는 1개월 이내에 대학에 이를 통보함.
6. 조선대학교는 저작물의 이용허락 이후 해당 저작물로 인하여 발생하는 타인에 의한 권리 침해에 대하여 일체의 법적 책임을 지지 않음
7. 소속대학의 협정기관에 저작물의 제공 및 인터넷 등 정보통신망을 이용한 저작물의 전송·출력을 허락함.

동의여부 : 동의(○) 반대( )

2010 년 8 월 일

저작자: 노 기 표 (서명 또는 인)

조선대학교 총장 귀하