저작자표시-비영리-변경금지 2.0 대한민국

이용자는 아래의 조건을 따르는 경우에 한하여 자유롭게

- 이 저작물을 복제, 배포, 전송, 전시, 공연 및 방송할 수 있습니다.

다음과 같은 조건을 따라야 합니다:

저작자표시. 귀하는 원저작자를 표시하여야 합니다.

비영리. 귀하는 이 저작물을 영리 목적으로 이용할 수 없습니다.

변경금지. 귀하는 이 저작물을 개작, 변형 또는 가공할 수 없습니다.

- 귀하는, 이 저작물의 재이용이나 배포의 경우, 이 저작물에 적용된 이용허락조건을 명확하게 나타내어야 합니다.
- 저작권자로부터 별도의 허가를 받으면 이러한 조건들은 적용되지 않습니다.

저작권법에 따른 이용자의 권리는 위의 내용에 의하여 영향을 받지 않습니다.

이것은 이용허락규약(Legal Code)을 이해하기 쉽게 요약한 것입니다.

Disclaimer
Comparative evaluation of biphasic calcium phosphate and biphasic calcium phosphate collagen composite on osteoconductive potency in rabbit calvarial defect

Dong-Ju Kim
Department of Dentistry
The Graduate School, Yonsei University
Comparative evaluation of biphasic calcium phosphate and biphasic calcium phosphate collagen composite on osteoconductive potency in rabbit calvarial defect

Directed by Professor Seong-Ho Choi

A Doctoral Dissertation submitted to the Department of Dentistry the Graduate School of Yonsei University in partial fulfillment of the requirements for the degree of Ph.D. in Dental Science

Dong-Ju Kim

September 2016
This certifies that the Doctoral Dissertation of Dong-Ju Kim is approved.

Thesis Supervisor: Seong-Ho Choi

Jung-Kiu Chai

Kyoo-Sung Cho

Chong-Kwan Kim

Ki-Tae Koo

The Graduate School
Yonsei University
December 2016
감사의 글

돌이켜보면 석사학위를 거쳐 박사학위를 받기까지 6 여 년의 시간이 흘렀습니다. 긴 시간이었지만 저에게는 순식간에 지나간 시간인 듯 합니다. 처음 대학원 과정을 시작할 때 최성호 교수님을 뵙고 지도를 받게 된 것이 제 인생에는 큰 행운이었습니다. 교수님께서 저를 끌어주시고 멀어주셔서 이 자리까지 오게 된 것이 아닌가 생각됩니다. 학문적인 가르침과 조언뿐만 아니라 인생에 있어서 어떤 삶을 살아야 하는 가에 대한 나침반 역할을 해주셨습니다. 물론 인품과 학문적 성취가 훌륭하신 김종관 교수님, 채중규 교수님, 조규성 교수님, 김창성 교수님, 정의원 교수님, 구기태 교수님, 이중석 교수님의 도움에도 진심으로 감사를 드립니다. 아울러 논문을 완성하는데 많은 역할을 해준 이은웅 선생님을 비롯한 치주과 의국원들께도 감사의 말씀을 드립니다.

모교에서 저에게 박사학위를 수여함은 제가 잘나서가 아니라 이제 모교의 명성을 드높이고 훌륭한 활동을 할 수 있는 자격이 되었음을 인정해주는 과정이라고 생각합니다. 앞으로 연세대학교 출신으로서 학문적 성취뿐만 아니라 사회에 공헌하고 존경받을 수 있는 한 인간으로 바로 설 수 있도록 더욱 정진하고 노력하겠습니다.

마지막으로 건강이 안 좋으신 저의 어머니의 건강이 회복되시길 간절히 기원하며, 그 옆에서 보살펴 주시는 저희 아버지, 제 후배이자 든든한 동료인 김영주 선생, 사랑하는 아내에게 감사함을 전합니다. 모든 분들이 항상 건강하시고 행복하시길…

2016 년 12 월
김 동 주
# Table of contents

List of Figures ........................................................................................................... ii
List of Tables .............................................................................................................. ii
Abstract (English) ....................................................................................................... iii
I. Introduction ............................................................................................................. 1
II. Materials and Methods ......................................................................................... 4
   1. Materials ............................................................................................................. 4
   2. Animals .............................................................................................................. 4
   3. Study design ..................................................................................................... 5
   4. Surgical procedure .......................................................................................... 5
   5. Histologic processing ..................................................................................... 6
   6. Analysis methods ............................................................................................. 6
III. Results .................................................................................................................... 8
   1. Clinical findings ............................................................................................... 8
   2. Histological Findings ..................................................................................... 8
   3. Histomorphometric Findings .......................................................................... 9
IV. Discussion .............................................................................................................. 13
V. Conclusion ............................................................................................................. 17
References ................................................................................................................ 18
Figure Legends ......................................................................................................... 24
Figures ..................................................................................................................... 25
Abstract (Korean) ..................................................................................................... 29
List of Figures

Figure 1. Four circular defects with a diameter of 8mm were made in the rabbit calvarium .................................................................25

Figure 2. Schematic diagram of histomorphometric analysis ..................26

Figure 3. Histologic transversal sections obtained 2 weeks after surgery ....27

Figure 4. Histologic transversal sections obtained 8 weeks after surgery ......28

List of Tables

Table 1. New bone area (mm2; Group Mean ± SD; n = 5) .........................11

Table 2. Defect closure (%; Group Mean ± SD; n = 5) ............................11

Table 3. Augmented area (mm2; Group Mean ± SD; n = 5) .......................12

Table 4. Residual material (mm2; Group Mean ± SD; n = 5) ....................12
Abstract

Comparative evaluation of biphasic calcium phosphate and biphasic calcium phosphate collagen composite on osteoconductive potency in rabbit calvarial defect

Dong-Ju Kim, D.D.S., M.S.D.

Department of Dentistry
The Graduate School, Yonsei University
(Directed by Professor Seong-Ho Choi, D.D.S., M.S.D., PhD.)

Background: The aim of this study was to determine the osteoconductivity of biphasic calcium phosphate collagen composite (BCPC) in rabbit calvarial defect model by comparing with biphasic calcium phosphate (BCP). Four 8-mm-diameter bicortical calvarial defects were made in ten rabbits. Each of the defects was randomly assigned and filled with 1) collagen sponge, 2) BCP, 3) BCPC, and 4) nothing as control. The animals were sacrificed at either 2 weeks (n=5) or 8 weeks (n=5) healing period.

Results: All groups showed wedge shaped new bone formation limited to the area of the defect margin at both healing periods. The amounts of new bone and defect
closure were similar among all groups. In the control and collagen sponge group, the center of the defect was depressed by surrounding tissues. In contrast, in BCP and BCPC group, the center of the defect did not depressed and the grafted materials maintained the space. And the augmented area was significantly higher in BCP and BCPC group compared to control and collagen sponge group at both healing periods (p < 0.05).

Conclusions: The BCPC and BCP demonstrated proper space maintaining capacity and osteoconductive property, suggesting BCPC can be efficiently utilized in various clinical situations.

KEYWORDS: Biphasic calcium phosphate, bone regeneration, bone substitutes, collagen, osteoconduction
Comparative evaluation of biphasic calcium phosphate and biphasic calcium phosphate collagen composite on osteoconductive potency in rabbit calvarial defect

Dong-Ju Kim, D.D.S., M.S.D.

Department of Dentistry
The Graduate School, Yonsei University
(Directed by Professor Seong-Ho Choi, D.D.S., M.S.D., PhD.)

I. Introduction

Various bone graft materials have been developed and used for reconstruction of bony defects in periodontal and implant surgeries. The autogenous bone is considered as the gold standard because of its superior capacity in bone formation, however, has some clinical drawbacks such as a morbidity of donor site and uncontrolled resorption rate [1,2]. Therefore, there have been many attempts to develop bone graft materials which can replace and substitute the autogenous bone.

Among various bone graft materials, calcium phosphate (Ca-P) bone substitutes
such as hydroxyapatite (HA) and beta-tricalcium phosphate (β-TCP) have been widely used because their chemical and structural characters are similar to those of human bone [1]. Indeed, they have shown favorable biocompatibility and osteoconductivity when used as bone graft materials [3]. Among Ca-Ps, HA, which is very stable, can maintain the space effectively but has low osteoconductivity [4,5]. In contrast, β-TCP is more biodegradable and rapidly replaced by newly formed bone but has low capacity of space maintaining [6]. Therefore biphasic calcium phosphate (BCP), which is composed of HA and β-TCP, was introduced to overcome limitations of each material and several studies have demonstrated that BCPs can be used as bone substitutes successfully [5,7,8].

Particle type BCPs have been frequently used and their clinical efficacy was well-demonstrated. However, there are some limitations on using particle type BCPs for bone substitutes. Particle type BCPs are susceptible to external compressive forces if not protected properly and easy to be collapsed in non-contained defects [9]. Therefore, the use of particle type BCPs may give technical difficulties to clinicians in specific situations. Hence, block type BCPs have been designed to impose stability in the defect of unfavorable. However, the application of block type BCPs requires adaptation, trimming and securement, which may increase possibilities of surgical error. Moreover, bone in-growth into the bone substitutes of block type depends much upon its structural properties, and some studies reported limited bone formation even though they were used with osteoinductive materials [9,10].

Recently, BCP collagen composite (BCPC) graft materials have been developed and
explored as bone substitutes. Due to excellent flexibility of the collagen, BCPC can be easily molded into the desired shape while maintaining the initial shape [11]. Moreover, collagen can promote healing process by retaining blood clots and inducing migration of fibroblasts [12]. Therefore BCPC can be expected to have more favorable biological and mechanical characteristics for bone formation and clinical handling, compared with above described BCPs. Thus, the aim of this study was to determine the osteoconductivity of BCPC in rabbit calvarial defect model by comparing with BCP.
II. Materials and Methods

1. Materials

The collagen sponges, BCP and BCPC were used as graft materials in this study. The collagen sponges were made of bovine type I collagen with cylinder form (8-mm-diameter and 2-mm-vertical height). The BCP (Osteon™ II, Genoss. Co. Ltd, Suwon, South Korea), a particle type graft material, was composed of HA and β-TCP with the weight ratio of 30/70. The sizes of particle and pore were 0.5 mm to 1.0 mm and 250㎛ respectively and the porosity was 70%. The BCPC (Osteon™ II collagen, Genoss. Co. Ltd, Suwon, South Korea) was block type graft material with cylinder form (8-mm-diameter and 2-mm-vertical height). It was composed of BCP (Osteon™ II, Genoss. Co. Ltd, Suwon, South Korea) and the bovine type I collagen and the weight percentage of the collagen was 4%.

2. Animals

Ten male New-Zealand white rabbits (mean body weight, 3.0-3.5kg; 14-16 weeks old) were used. All animals were housed in separate cages with standard laboratory conditions and fed with standard diets. All of the procedures including animal selection, management, preparation and surgical procedures followed protocols approved by the Institutional Animal Care and Use Committee, Yonsei Medical
3. Study design

In each rabbit calvarium, four circular bi-cortical defects with a diameter of 8 mm were formed. And then, the defects were randomly assigned to one of the following four experimental groups and filled with each material (Fig. 1). The rabbits were allowed to get healing period of 2 weeks (n=5) or 8 weeks (n=5).

- Control group: The defect was filled with blood clot only.
- Collagen group: The collagen sponge was filled in the defect.
- BCP group: BCP was filled in the defect.
- BCPC group: BCPC was filled in the defect.

4. Surgical procedure

The animals were intramuscularly injected and anesthetized with a mixture of Ketamine hydrochloride (KetalarVR, Yuhan Co, Seoul, Korea) and Xylazine (RumpunVR, Bayer Korea Ltd, Seoul, Korea). After that, the surgical sites were shaved and draped with povidone iodine and alcohol followed by local anesthesia with 2% lidocaine. An incision along the sagittal midline from frontal bone to occipital bone was made and full thickness flap elevation was performed. Under the saline irrigation, four circular bi-cortical defects with a diameter of 8 mm were made by using a trephine bur. Then, randomly assigned graft materials were grafted into the defects. The flap was sutured with a biodegradable suture material (Monosyn, B-
Braun, Melsungen, Germany) which was removed 10 days later. Five animals were
sacrificed at 2 and 8 weeks of post surgery.

5. Histologic processing

Before embedding in paraffin blocks, all sections were fixed using 10% buffered
formalin solution and decalcified with 5% nitric acid (nitric acid, Daejung Chemicals
& Metals Co., Kyonggi, Korea). Then, serial sectioning with 5㎛ were performed
along the sagittal derection of the rabbit calvarium. Two sections closest to the center
of the defect were chosen and stained with hematoxylin and eosin and Goldner’s
Masson trichrome.

6. Analysis methods

Clinical and histologic observations For the clinical observation, animals were
carefully observed and evaluated for the presence of allergic reaction, exposure of
graft materials and inflammatory reaction at 2 and 8 weeks after the surgery. For the
histological observation, a binocular microscope (Leica DM LB, Leica Microsystems
Ltd., Wetzlat, Germany) was used and all of the specimens were examined by a single
blinded examiner.

Histomorphometric observations For evaluating the biodegradation and bone
healing, following four histomorphometric parameters were measured using an
automated image-analysis system (Image-Pro Plus, Media Cybernetics, Silver Spring, MD, USA) (Fig. 2).

1. New bone area: the area of newly formed bone in the defect
2. Defect closure: the ratio of the distance between the newly formed bone over the initial distance between the original defect margin
3. Augmented area: the area between the defect margins including bone tissues, marrow tissues, residual materials, fibrovascular tissues and adipose tissues
4. Residual material: the area of remaining grafted materials in the defect

**Statistical analysis** All values were presented as mean ± standard deviation and a commercial software program (SPSS 21.0, SPSS Inc., Chicago, IL, USA) was used for the statistical analysis. For multiple comparisons among the groups in a given time, one-way ANOVA method test and Tukey-HSD post hoc procedure were performed. Independent t-test was carried out to analyze the statistical difference between 2 and 8 weeks healing period within each group.
III. RESULTS

1. Clinical findings

All experimental sites showed uneventful healing during the postoperative healing period. Clinically, there was no specific evidence of complications such as exposure of graft materials.

2. Histological findings

2 weeks healing period All groups showed small amount of wedge shaped new bone formation limited to the areas of the defect margin and the amounts of newly formed bone seemed to be similar among all groups. In the control and collagen sponge group, the center of the defect was depressed and flattened by surrounding connective tissue and dura mater. In contrast, in BCP and BCPC group, the center of the defect was not depressed and the grafted materials maintained the space (Fig. 3a, 3c, 3e, 3g).

On high magnification view, in the collagen sponge group, the grafted collagen sponge was resorbed and collapsed. In BCP and BCPC group, at the border of newly formed bone, osteoblasts and osteoids were found in close contact with BCP particles and inter-granular spaces were mainly filled with loose connective tissue. The collagen matrix of BCPC could not be clearly distinguished from inter-particular
connective tissues of BCP (Fig. 3b, 3d, 3f, 3h).

8 weeks healing period The histological findings of the control and collagen sponge group were similar at 8 weeks healing period. In the control and collagen sponge group, more newly formed bone were found around the defect margin compared to those of 2 weeks healing period. However, the center of the defect was collapsed and mainly filled with loose connective tissue. Among few specimens, the bony islands were found at the center of the defect (Fig. 4a, 4b, 4c, 4d). In BCP and BCPC group, the sizes of BCP granules were similar between two groups, and reduced compared to those of 2 weeks healing period. The space between defect margins was maintained by grafted BCP particles. However, most of the newly formed bone was limited to the defect margin, and loose connective tissue and BCP particles occupied the space at the center of the defect (Fig. 4e, 4f, 4g, 4h).

3. Histomorphometric Findings

The histomorphometric measurements are summarized in Table 1, 2, 3 and 4. There was no statistically significant difference in new bone area and defect closure among all groups during the same healing period. All of the groups, except the collagen sponge group, showed statistically significant increase of new bone formation between 2 and 8 weeks healing period. In terms of defect closure, the values significantly increased between 2 and 8 weeks healing periods in all of the groups.
(Table 1, 2).

The augmented area was statistically greater in BCP and BCPC group than those of the control and the collagen sponge group at 2 and 8 weeks healing periods. There was no statistically significant difference between 2 and 8 weeks healing periods in the same experimental group (Table 3).

The amount of residual material was similar between BCP and BCPC group at the same healing period and decreased between 2 and 8 weeks healing periods (Table 4).
Table 1 New bone area (mm$^2$; Group Mean ± SD; n = 5)

| Group   | 2 weeks  | 8 weeks  |
|---------|----------|----------|
| Control | 1.24±0.35| 3.29±0.62*|
| Collagen| 1.66±0.85| 2.43±0.44 |
| BCP     | 1.33±0.49| 2.47±0.25*|
| BCPC    | 1.32±0.56| 2.41±0.20*|

There was no statistically significant difference among all groups in same healing period.

* Significantly different from same experimental group at 2 weeks.

Table 2 Defect closure (%; Group Mean ± SD; n = 5)

| Group | 2 weeks  | 8 weeks  |
|-------|----------|----------|
| Control| 29.25±4.61| 54.76±7.44*|
| Collagen| 29.84±5.97| 48.78±6.96*|
| BCP   | 26.29±5.73| 50.95±3.66*|
| BCPC  | 26.52±4.64| 45.75±11.62*|

There was no statistically significant difference among all groups in same healing period.

* Significantly different from same experimental group at 2 weeks.
Table 3 Augmented area (mm$^2$; Group Mean ± SD; n = 5)

|          | 2 weeks     | 8 weeks     |
|----------|-------------|-------------|
| Control  | 4.37±0.65   | 8.00±0.79   |
| Collagen | 5.06±2.34   | 5.59±0.84   |
| BCP      | 12.88±2.45* | 14.23±0.71* |
| BCPC     | 14.35±0.91* | 12.92±2.56* |

* Significantly different from control and collagen group in same healing period.

Table 4 Residual material (mm$^2$; Group Mean ± SD; n = 5)

|        | 2 weeks     | 8 weeks     |
|--------|-------------|-------------|
| Collagen | 0           | 0           |
| BCP     | 3.89±0.83   | 2.66±0.43*  |
| BCPC    | 4.24±0.41   | 2.09±0.63*  |

* Significantly different from same experimental group at 2 weeks.
IV. DISCUSSION

Biologically, the optimal bone substitutes must possess biocompatibility, osteoconductivity, osteoniductivity and similar structure to human bone. They should provide structural framework for blood clot stabilization, maturation, and replacement space for newly formed bone [2,13]. In a clinical point of view, easy handling and cost-effectiveness are also important. Hence, many researches have been performed in a pursuit of both respects.

In the present study, we evaluated the osteoconductivity of BCPC in the rabbit calvarial defect. These days, the addition of collagen to bone substitute is expected to give benefits in bone regenerative therapy of dental field, on which many studies are focused. Collagen, which is one of the major organic components of human bone, has several advantageous properties for bone healing including hemostasis, chemotactic activity to attract fibroblast, and promotion of wound stabilization [14-16]. Moreover, it has been proven that collagen regulates gene expression and differentiation of osteoclasts [17]. Nonetheless, collagen itself has a major drawback as a bone substitute due to fast resorption by the activity of macrophages, leukocytes and bacteria, resulting in failure of space maintenance for new bone in-growth [15,18].

However, addition of collagen to bone substitute may compensate for the weaknesses of conventional bone substitutes. Particle type BCPs have been used in many studies and demonstrated space maintaining capacity and bone regenerative potential [8,19,20], but the performance of BCP on bone regeneration may be
compromised in non-contained defects [9]. Block type BCP may be advantageous to overcome unfavorable defects, but its application may entail surgical difficulty and its three-dimensional microstructure may not be appropriate for bone in-growth [9,10]. With combination of BCP and collagen, BCP particles can provide volume stability and collagen matrix can promote cell proliferation and infiltration of osteoblasts [21].

Rabbit calvarial defect models have been used widely for evaluating newly developed bone substitutes because of adequate quantity and quality of bone marrow [22]. The critical size of rabbit calvarial defect has been considered as 10-15mm through several studies [23-25]. Although 8-mm-diameter rabbit calvarial defect is smaller than critical one, it has been known to be useful to evaluate defect healing pattern and bone regeneration [26,27]. And it has been proven that 8mm circular defects in rabbit calvarium show limited bone healing within 8 weeks healing period in several studies [28,29]. Therefore, in this study, four 8mm circular defects were made in the rabbit calvarium and bone healing was evaluated at 2 and 8 weeks after surgery, respectively. There could be a concern that extravasation of experimental materials from neighboring defects affected the results. However, the type of defect used in this study was contained. And no graft materials from another defect were found in the control site. Therefore the effect of neighboring defects can be thought to be minimal.

In histologic and histomorphometric analysis, collagen sponge was totally resorbed and collapsed within 2 weeks healing period and showed similar healing pattern to control group. However, BCP and BCPC showed relatively slow resorption and
maintained the space effectively, which is consistent with other previous studies [30,31]. This result suggests that both BCP and BCPC have favorable space maintaining capacity. The maintenance of the space for bone in-growth is important to stabilize blood clots and prevent downward growth of epithelium [32,33].

The amount of new bone and defect closure increased throughout the healing period in all groups. And there was no statistically significant difference among all groups at 2 and 8 weeks healing periods. Although BCP graft materials are commonly used as bone substitutes, the capacity of new bone formation of BCPs remains controversial. Numerous studies have demonstrated enhanced bone formation when BCPs were grafted in various defect models [7,29,30]. Whereas, some studies reported that grafted BCPs did not induce increased bone formation, which coincides with this study [31,34]. Therefore recent studies have tried to enhance the osteogenic capacity of BCP graft materials using osteoinductive materials such as BMPs [10,35]. Although increased new bone formation did not occur in this study, new bone was found in close contact with BCP particles in BCP and BCPC group with high magnification observation. This means that both BCP and BCPC are biocompatible and have good osteoconductive properties.

In this study, BCP and BCPC exhibited similar healing and bone regeneration pattern in total amount of new bone and augmented area. Both materials could be used as promising bone substitutes successfully. Further studies should be carried out to confirm that BCPC has advantages over BCP in the healing of non-contained type defects.
Collagen which is a component of BCPC has been widely used as a carrier of bioactive materials [36]. In a previous study, it has been shown that BCPC can be soaked with growth factors easily [37]. Therefore, BCPC could be also used as a promising carrier when bony defects are large or non-contained form where space maintenance becomes highly important.
V. CONCLUSION

In conclusion, the BCPC and BCP demonstrated proper space maintaining capacity and osteoconductive property, suggesting BCPC can be efficiently utilized in various clinical situations.
REFERENCES

1. E. M. Erbe, J. G. Marx, T. D. Clineff and L. D. Bellincampi, "Potential of an ultraporous beta-tricalcium phosphate synthetic cancellous bone void filler and bone marrow aspirate composite graft," European spine journal : official publication of the European Spine Society, the European Spinal Deformity Society, and the European Section of the Cervical Spine Research Society, 10 Suppl 2 S141-6 (2001).

2. E. Rosenberg and L. F. Rose, "Biologic and clinical considerations for autografts and allografts in periodontal regeneration therapy," Dental clinics of North America, 42(3) 467-90 (1998).

3. T. Han, F. A. Carranza, Jr. and E. B. Kenney, "Calcium phosphate ceramics in dentistry: a review of the literature," The Journal of the Western Society of Periodontology/Periodontal abstracts, 32(3) 88-108 (1984).

4. B. S. Moskow and A. Lubarr, "Histological assessment of human periodontal defect after durapatite ceramic implant. Report of a case," Journal of periodontology, 54(8) 455-62 (1983).

5. R. F. Ellinger, E. B. Nery and K. L. Lynch, "Histological assessment of periodontal osseous defects following implantation of hydroxyapatite and biphasic calcium phosphate ceramics: a case report," The International journal of periodontics & restorative dentistry, 6(3) 22-33 (1986).

6. S. V. Dorozhkin, "Bioceramics of calcium orthophosphates," Biomaterials, 31(7) 1465-85 (2010).

7. G. Daculsi, N. Passuti, S. Martin, C. Deudon, R. Z. Legeros and S. Raher,
"Macroporous calcium phosphate ceramic for long bone surgery in humans and dogs. Clinical and histological study," Journal of biomedical materials research, 24(3) 379-96 (1990).

8. E. B. Nery, R. Z. LeGeros, K. L. Lynch and K. Lee, "Tissue response to biphasic calcium phosphate ceramic with different ratios of HA/beta TCP in periodontal osseous defects," Journal of periodontology, 63(9) 729-35 (1992).

9. J. W. Kim, K. H. Choi, J. H. Yun, U. W. Jung, C. S. Kim, S. H. Choi and K. S. Cho, "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 surgery, oral medicine, oral pathology, oral radiology, and endodontics, 112(3) 298-306 (2011).

10. J. W. Kim, I. H. Jung, K. I. Lee, U. W. Jung, C. S. Kim, S. H. Choi, K. S. Cho and J. H. Yun, "Volumetric bone regenerative efficacy of biphasic calcium phosphate-collagen composite block loaded with rhBMP-2 in vertical bone augmentation model of a rabbit calvarium," Journal of biomedical materials research. Part A, 100(12) 3304-13 (2012).

11. M. G. Patino, M. E. Neiders, S. Andreana, B. Noble and R. E. Cohen, "Collagen as an implantable material in medicine and dentistry," The Journal of oral implantology, 28(5) 220-5 (2002).

12. T. Twardowski, A. Fertala, J. P. Orgel and J. D. San Antonio, "Type I collagen and collagen mimetics as angiogenesis promoting superpolymers," Current pharmaceutical design, 13(35) 3608-21 (2007).

13. H. F. Nasr, M. E. Aichelmann-Reidy and R. A. Yukna, "Bone and bone substitutes," Periodontology 2000, 19 74-86 (1999).
14. A. E. Postlethwaite, J. M. Seyer and A. H. Kang, "Chemotactic attraction of human fibroblasts to type I, II, and III collagens and collagen-derived peptides," Proceedings of the National Academy of Sciences of the United States of America, 75(2) 871-5 (1978).

15. D. Rothamel, F. Schwarz, M. Sager, M. Herten, A. Sculean and J. Becker, "Biodegradation of differently cross-linked collagen membranes: an experimental study in the rat," Clinical oral implants research, 16(3) 369-78 (2005).

16. F. Schwarz, D. Rothamel, M. Herten, M. Sager and J. Becker, "Angiogenesis pattern of native and cross-linked collagen membranes: an immunohistochemical study in the rat," Clinical oral implants research, 17(4) 403-9 (2006).

17. M. P. Lynch, J. L. Stein, G. S. Stein and J. B. Lian, "The influence of type I collagen on the development and maintenance of the osteoblast phenotype in primary and passaged rat calvarial osteoblasts: modification of expression of genes supporting cell growth, adhesion, and extracellular matrix mineralization," Experimental cell research, 216(1) 35-45 (1995).

18. M. N. Sela, D. Kohavi, E. Krausz, D. Steinberg and G. Rosen, "Enzymatic degradation of collagen-guided tissue regeneration membranes by periodontal bacteria," Clinical oral implants research, 14(3) 263-8 (2003).

19. B. H. Fellah, O. Gauthier, P. Weiss, D. Chappard and P. Layrolle, "Osteogenicity of biphasic calcium phosphate ceramics and bone autograft in a goat model," Biomaterials, 29(9) 1177-88 (2008).

20. K. B. Fleckenstein, M. F. Cuenin, M. E. Peacock, M. A. Billman, G. D. Swiec, T. B. Buxton, B. B. Singh and J. C. McPherson, 3rd, "Effect of a hydroxyapatite tricalcium phosphate alloplast on osseous repair in the rat calvarium," Journal of
periodontology, 77(1) 39-45 (2006).

21. J. C. Brodie, J. Merry and M. H. Grant, "The mechanical properties of calcium phosphate ceramics modified by collagen coating and populated by osteoblasts," Journal of materials science. Materials in medicine, 17(1) 43-8 (2006).

22. E. Newman, A. S. Turner and J. D. Wark, "The potential of sheep for the study of osteopenia: current status and comparison with other animal models," Bone, 16(4 Suppl) 277S-84S (1995).

23. P. Pripatnanont, T. Nuntanaranont and S. Vongvatcharanon, "Proportion of deproteinized bovine bone and autogenous bone affects bone formation in the treatment of calvarial defects in rabbits," International journal of oral and maxillofacial surgery, 38(4) 356-62 (2009).

24. S. Xu, K. Lin, Z. Wang, J. Chang, L. Wang, J. Lu and C. Ning, "Reconstruction of calvarial defect of rabbits using porous calcium silicate bioactive ceramics," Biomaterials, 29(17) 2588-96 (2008).

25. J. M. Shand, A. A. Heggie, A. D. Holmes and W. Holmes, "Allogeneic bone grafting of calvarial defects: an experimental study in the rabbit," International journal of oral and maxillofacial surgery, 31(5) 525-31 (2002).

26. D. Lundgren, S. Nyman, T. Mathisen, S. Isaksson and B. Klinge, "Guided bone regeneration of cranial defects, using biodegradable barriers: an experimental pilot study in the rabbit," Journal of cranio-maxillo-facial surgery : official publication of the European Association for Cranio-Maxillo-Facial Surgery, 20(6) 257-60 (1992).

27. S. C. Cavalcanti, C. L. Pereira, R. Mazzonetto, M. de Moraes and R. W. Moreira, "Histological and histomorphometric analyses of calcium phosphate cement in rabbit calvaria," Journal of cranio-maxillo-facial surgery : official publication of the
European Association for Cranio-Maxillo-Facial Surgery, 36(6) 354-9 (2008).

28. J. Y. Sohn, J. C. Park, Y. J. Um, U. W. Jung, C. S. Kim, K. S. Cho and S. H. Choi, "Spontaneous healing capacity of rabbit cranial defects of various sizes," Journal of periodontal & implant science, 40(4) 180-7 (2010).

29. C. Yang, O. Unursaikhan, J. S. Lee, U. W. Jung, C. S. Kim and S. H. Choi, "Osteoconductivity and biodegradation of synthetic bone substitutes with different tricalcium phosphate contents in rabbits," Journal of biomedical materials research. Part B, Applied biomaterials, 102(1) 80-8 (2014).

30. H. C. Lim, J. Y. Sohn, J. C. Park, Y. J. Um, U. W. Jung, C. S. Kim, Y. K. Lee and S. H. Choi, "Osteoconductive effects of calcium phosphate glass cement grafts in rabbit calvarial defects," Journal of biomedical materials research. Part B, Applied biomaterials, 95(1) 47-52 (2010).

31. L. H. Park JC, Sohn JY, Yun JH, Jung UW, Kim CS et al, "Bone regeneration capacity of two different macroporous biphasic calcium materials in rabbit calvarial defect.," J Korean Acad Periodontol, 2009;39:223–230.

32. C. Dahlin, A. Linde, J. Gottlow and S. Nyman, "Healing of bone defects by guided tissue regeneration," Plastic and reconstructive surgery, 81(5) 672-6 (1988).

33. S. Nyman, J. Lindhe, T. Karring and H. Rylander, "New attachment following surgical treatment of human periodontal disease," Journal of clinical periodontology, 9(4) 290-6 (1982).

34. L. A. Strobel, S. N. Rath, A. K. Maier, J. P. Beier, A. Arkudas, P. Greil, R. E. Horch and U. Kneser, "Induction of bone formation in biphasic calcium phosphate scaffolds by bone morphogenetic protein-2 and primary osteoblasts," Journal of tissue engineering and regenerative medicine, 8(3) 176-85 (2014).
35. Y. S. Shin, J. Y. Seo, S. H. Oh, J. H. Kim, S. T. Kim, Y. B. Park and H. S. Moon, "The effects of ErhBMP-2-/EGCG-coated BCP bone substitute on dehiscence around dental implants in dogs," Oral diseases, 20(3) 281-7 (2014).

36. M. Geiger, R. H. Li and W. Friess, "Collagen sponges for bone regeneration with rhBMP-2," Advanced drug delivery reviews, 55(12) 1613-29 (2003).

37. W. Friess, H. Uludag, S. Foskett, R. Biron and C. Sargeant, "Characterization of absorbable collagen sponges as recombinant human bone morphogenetic protein-2 carriers," International journal of pharmaceutics, 185(1) 51-60 (1999).
Figure Legends

Figure 1. Four circular defects with a diameter of 8mm were made in the rabbit calvarium and the experimental materials were grafted as follows: (1) no graft, (2) Collagen sponge, (3) BCP, (4) BCPC

Figure 2. Schematic diagram of histomorphometric analysis.

Figure 3. Histologic transversal sections obtained 2 weeks after surgery: (a, b) control group, (c, d) collagen sponge group, (e, f) BCP group, (g, h) BCPC group. Arrowheads = defect margin; NB = new bone; G = graft material; C = connective tissue. (a, c, e, g) Goldner’s Masson trichrome stain and original magnification: 40X; (b, d, f, h) Hematoxylin and eosin stain and original magnification: 200X.

Figure 4. Histologic transversal sections obtained 8 weeks after surgery: (a, b) control group, (c, d) collagen sponge group, (e, f) BCP group, (g, h) BCPC group. Arrowheads = defect margin; NB = new bone; G = graft material; C = connective tissue. (a, c, e, g) Goldner’s Masson trichrome stain and original magnification: 40X; (b, d, f, h) Hematoxylin and eosin stain and original magnification: 200X.
Figures

Figure 1.
Figure 2.
Figure 3.
Figure 4.
국문요약

토끼 두개골 결손부에서 이상인산칼슘과 이상인산칼슘 콜라겐 복합체의 골전도능 비교 평가

＜지도교수 최성호＞
연세대학교 대학원 치의학과
김동주

여러 가지 골대체제 중 이상인산칼슘은 그 화학적, 구조적 특성이 인간 골과 유사하여 널리 쓰이고 있다. 이상인산칼슘 성분 중 하나인 수산화회석은 공간유지능력은 뛰어나나 낮은 골전도성을 보이는 반면에 제삼인산칼슘은 빠르게 흡수되어 신생골로 대체된다.

입자 형태의 이상인산칼슘은 여러 임상 상황에서 널리 쓰이거나 외력에 취약하여 쉽게 붕괴될 수 있다. 그리하여 블록 형태의 이상인산칼슘이 안정성을 높이기 위해 개발되었으나 적합시키기 어렵고, 신생골이 자라들어 가기가 어렵다는 단점이 있다.

이런 제약을 극복하기 위해 최근에 소개된 이상인산칼슘 교원질 복합체는 조작성이 좋으며, 교원질이 초기 치유에 도움이 될 것으로 기대된다.
따라서 이 연구의 목적은 토끼 두개골 결손 모델에서 이상인산칼슘과 이상인산칼슘 교원질 복합체의 골전도능을 비교 평가하는 것이다.

10마리의 토끼 두개골에 직경 8mm의 원형 결손부를 4개씩 형성하여 한 결손부는 대조군으로써 그대로 두고 나머지 세 결손부위에는 각각 교원질 해면체, 이상인산칼슘, 이상인산칼슘 교원질 복합체를 무작위로 이식하였다. 그 후 2주와 8주 후에 5마리씩 희생하여 조직학적, 조직형태학적 분석을 하였다.

2주와 8주의 치유 기간 동안 모든 군에서 결손 경계 부위에 쐐기 모양의 신생골 형성이 관찰되었다. 신생골의 양과 결손부위 피개 정도는 모든 군에서 비슷하였다. 대조군과 교원질 해면체 군에서는 결손부위 중심이 주변 조직에 의해 함몰되어 있는 반면에 이상인산칼슘과 이상인산칼슘 교원질 복합체 군에서는 함몰되어 있지 않았으며, 이식재에 의해 공간이 유지되고 있었다. 또한 대조군과 교원질 해면체 군에 비해 조직 증대 면적이 이상인산칼슘과 이상인산칼슘 교원질 복합체 군에서 통계적으로 유의하게 높았다.

이상의 결과로 볼 때 이상인산칼슘과 마찬가지로 이상인산칼슘 교원질 복합체도 적절한 공간유지능력과 골전도능을 보여 다양한 임상상황에 효과적으로 사용될 수 있으리라 여겨진다.

핵심되는 말: 이상인산칼슘, 골재생, 골대체제, 교원질, 골전도