The Effects of Calcium-Phosphate Coated Xenogenic Bone and Type I Collagen for Bone Regeneration on the Calvarial Defects in Rabbits

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I. Introduction

Bone grafts have long been used in reconstructive surgery with the aim of increasing the bone volume in the previous defect area. Bone grafts and bone substitute materials may be classified into two main groups : autogenic and xenogenic materials. The term autogenic graft refers to tissues that are transplanted within one and the same organism. Xenogenic grafts encompass all materials of an origin other than the recipient's organism and may further be divided into materials from the same species but different individual, materials from other species, and finally products of non-organic origin[1].

An ideal bone graft or bone substitute material should presumably have the following characteristics that are sterile, not eliciting any immunological reaction, osteoconductive or osteoinductive, favorable clinical handling, resorbed and replaced by bone, available in sufficient quantities, and low in cost[2].

The autogenous bone graft is the most predictable material that possesses both osteoconductive and osteoinductive properties[1,3]. However, in an attempt to avoid separate surgical procedures involving remote donor site and reduce postsurgical pain, patient inconvenience, operating time and cost, clinicians have increased their use of alternative grafting materials.

Demineralized freeze-dried bone allograft(DFDBA) has been used as a bone graft substitute in periodontal therapy for the last 2 decades[4]. DFDBA has been used successfully to regenerate bone as well as cementum in the treatment of periodntal defects[5-8] and to regenerate bone around implants[9-11] and for localized alveolar ridge augmentation[12]. However, more recent experiments have questioned the ability of DFDBA to induce new bone formation[13-17]. Also, DFDBA has a small but measurable risk of disease transmission. Although the risk of disease is low if materials are processed correctly, many patients are reluctant to provide consent due to fear of disease[18].

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Several materials have been used in periodontics and oral and maxillofacial surgery as alternatives to DFDBA and autogenous bone graft. These materials including tricalcium phosphate[19], calcium sulfate[20], polymers[21] and bioactive glass[22] generally act as biocompatible soft tissue fillers and have relatively few osteogenic properties.

So, considerable effort has been directed toward development of osseous implant materials that are readily available, osteoconductive, biocompatible and similar in structure to human bone. In an effort to use a synthetic material that closely approximates the bioapatite found in bone, products have been developed that are derived from bovine bone. As the material has no organic substances, deproteinized cancellous bovine bone has been considered to be safe as a xenograft material. At the same time, the chemical and physical properties of natural bone mineral are retained. The surface area of each graft particle is considerably greater than that of porous synthetic bioceramics, and the modulus of elasticity is similar to that of natural bone.

The biocompatibility of this bone graft was demonstrated by Denissen et al.[23] and Cohen et al.[24] Denissen et al.[23] concluded that natural bone minerals are fully compatible with the tibia of the rat and that no degradation of the implant material occurred for intervals of up to 6 months after implantation. Cohen et al.[24] measured inflammatory changes associated with implantation of anorganic bovine bone. As a result, a systemic or local immune response did not develop following implantation with natural bone mineral derived bovine bone in the rats over 3 days and 1, 2, 4, 6, and 8 weeks periods of healing. This material has been shown clinically and histologically to be an effective biocompatible, osteoconductive filler with degradable capacity[25,26].

In experimental and clinical studies, as interests about xenogenic bone were increasing, a new type of xenogenic grafting materials(BBPII™®) derived from bovine bone was recently developed and used clinically. The surface of this material was coated with calcuim-phosphate(Ca-P) nanocrystal thin film for superior regenerative ability. The fat and organic components of bovine cancellous were removed completely to eliminate immunologic reaction. Furthermore, it is sterilized with radiation after packaging, so as to not have toxic remnant as in sterilization with ethylene oxide gas. A few histologic studies reported it was effective on furcation involvement[27] and extraction socket[28] of dogs. But, the effect of Ca-P coated xenogenic bone has not been extensively investigated.

Particulate xenogenic bone grafts used to fill a bony defect have a tendency to migrate after placement, even with careful surgical techniques. Due to the difficulty in confining implant particles, Harvey et al.[29] suggested that collagen might be used as a biocompatible resorbable binder to prevent migration of the particulate bone materials until they are incorporated into the patient's tissue. Gongloff et al.[30] and Shen et al.[31] reported that collagen tubes prevent migration of hydroxyapatite particles.

The objective of this study was to assess the osseous responses to particles of Ca-P coated xenogenic bone derived from bovine bone and to compare the osteogenic potential of these particles with that of a combination with type I collagen derived from bovine tendon as a biocompatible binder to prevent migration of the particulate bone materials.

II. Materials and Methods

1. Animals

Sixteen adult male New Zealand white rabbits

Table 1 Experimental protocol

	Group 1	Group 2	Group 3	Group 4
Experimental material used	control	auto	BBPII	BBPII + col I
Experimental site	frontal bone	frontal bone	parietal bone	parietal bone
Total no. of animals (observation periods : 1,2,4 and 8 weeks)	16	16	16	16
No. of animal excluded as the result of death	0	0	0	0

Control = nongrafted control group ; auto = autologous bone ; col I = type I collagen

weighing 3.0 to 3.5kg were used in this study. They were kept in standard laboratory conditions of a light-dark schedule and relative humidity and fed a standard rabbit diet and maintained in separate cages. Four animals were killed by a heart perfusion after anesthesia at 1 week, four at 2 weeks, four at 4 weeks and four at 8 weeks after surgery.

2. Experimental materials

Calcium-phosphate(Ca-P) coated resorbable natural bone mineral(BBPII™®, particle size. 0.4 to 0.6mm; OCT Inc. Chonan Korea) and type I collagen from bovine achilles tendon(Sigma chemical Co, USA) were used in the study.

3. Surgical procedure

The animals were anesthetized preoperatively with an intramuscular injection of ketamine hydrochloride 44mg/kg of body weight (Ketara®, Yuhan Corporation, Seoul Korea) and xylazine 7mg/kg of body weight (Rumpum®, Bayer korea. Seoul Korea). The dorsal part of the cranium was shaved and prepared aseptically for surgery and 2% Lidocaine (contained epinephrine 1:80,000, Yuhan, pharm, Korea) were injected for local anesthesia and bleeding control in the midline of the cranium. A thirty miliimeter long incision in the scalp along the sagittal suture was made. Skin, musculature and periosteum were reflected and the parietal bones and frontal bones were exposed. Four full thick skull defects were made in the frontal and parietal bones with a 5-mm trephine bur. A 5-mm trephine bur was used to create the defects under copious irrigation with sterile physiologic saline to prevent overheating of the bone edges. The circular bone plugs were gently removed and extreme care was exercised to avoid injury to the duramater and its fibrous attachments to the inner table of the calvarial bone. The bone plugs were crushed several times with pliers for later use. The defects were rinsed with sterile saline and then either filled with implants or left empty. The periosteum was repositioned and the incision was closed. The defects were evaluated at 1, 2, 4 and 8 weeks after implantation. Four experimental groups were constructed(Table 1) : Group 1, nongrafted control defects; group 2, defects filled with autogenous bone chip; group 3, defects filled with Ca-P coated resorbable natural bone mineral(BBPII™®); group 4, defects filled with Ca-P coated resorbable natural bone mineral and type I collagen (1:1 mixture by volume). After the surgery, each animal was injected intramuscularly with antibiotics(Baytril® Bayer Korea, Seoul Korea) at a dose of 0.2ml/kg and analgesics (Nobin® Bayer Korea, Seoul Korea) at a dose of 0.44mg/kg once daily for 1 week.

4. Histologic evaluation

All animals were sacrificed by heart perfusion and specimens were taken at the site of frontal and parietal bone around the calvarial defect areas. Specimens from four experimental groups were fixed with the mixture of 4% paraformaldehyde in 0.1M phosphate buffered saline(PBS). After demineralization with 10% EDTA, the specimens were dehydrated with a graded series of ethanol, embedded in paraffin, sectioned at 5㎛ with microtome, and stained with hematoxylin and eosin (H-E) and Masson's trichrome.

III. Results

1. Clinical observation

All animals remained healthy during the observation period, and all sites of implantation healed uneventfully. There were no signs of infection, edema or extrusion of implant materials.

2. Histologic observation

1) Nongrafted control defects (Group 1)

At 1 week, contaminating focal hemorrhagic area was seen at defects with mild infiltration of inflammatory cells. Limited immature bone trabeculae was found at the margin of the defects(Figure 1, 2, 3 & 4). After 2 weeks, newly formed bone completely bridged the defect margins. Connective tissue with rich blood vessels was filled between trabeculae. No evidence of inflammatory response was found(Figure 5, 6, 7 & 8). At 4 weeks compared with specimens at 2 weeks after surgery, the new bone was thicker and more matured(Figure 9, 10, 11 & 12). At 8 weeks, the defects were regenerated into mature bone with marrow space but not attained a thickness similar to that of the surrounding bone(Figure 13, 14, 15 & 16).

2) Defects filled with autogenous bone chip (Group 2)

At 1 week, the defects were filled with granulation tissue in which mild inflammatory cell infiltration and hemorrhage were found. At surrounding the remaining bone graft particle as well as at the margin of bone defect, a formation of immature bone trabeculae was observed(Figure 17, 18, 19 & 20). After 2 weeks, infiltration of inflammatory cells was not noted, and regenerated bone had attained a thickness similar to that of the surrounding bone. The grafted bone and a new growing bone was in direct union without soft tissue intervention. Loose connective tissue with blood vessels was found between trabeculae(Figure 21, 22, 23 & 24). At 4 weeks, the regenerated bone was more mature than specimens at 2 weeks(Figure 25, 25, 27 & 28). Defects were completely regenerated into mature bone with homogeneous thickness at 8 weeks(Figure 29, 30, 31 & 32).

3) Defects filled with Ca-P coated resorbable natural bone mineral (Group 3)

At 1 week, inflammatory cells were infiltrated around graft materials. The defects were occupied with thicker loose connective tissue than those of the nongrafted group. New bone formation was found at the margin of the defects. Partially, it was in union with graft material without intervention of soft tissue. Some of the graft materials were resorbed by multinucleated giant cells(Figure 33, 34, 35 & 36). After 2 weeks, hemorrhage and granulation tissue were seen and a moderate inflammatory response was partially noted. New bone trabeculae was detected at central part of the defects as well as the margin. Newly formed bone was seen in direct apposition to the bone particles. Bone graft was

resorbed by multinucleated giant cells in some parts(Figure 37, 38, 39 & 40). At 4 weeks, there were chronic inflammation and bleeding. The connective tissue around the graft materials had many cellular components . Partly active bone formation was noted. Direct union between the graft material and new bone was not detected. Some graft materials at the cental region was absorbed by multinucleated giant cells(Figure 41, 42, 43 & 44). Chronic inflammation was found in the connective tissue, and absorption by multnucleated giant cell activity was sustained at 8 weeks. New bone formation was seen partially, but mainly around periosteum. No direct union with the graft material was detected(Figure 45, 46, 47 & 48).

4) Defects filled with Ca-P coated resorbable natural bone mineral and type I collagen (Group 4)

At 1 week, there was a marked inflammatory mononuclear cell infiltration around the collagen and Ca-P coated xenogenic bone particle. New bone formation around the established bone was seen, but not around the graft material. Linear collagen was found in part, and multinucleated giant cells were detected around the graft material(Figure 49, 50, 51 & 52). By 2 weeks, infiltration of inflammatory cells was reduced, similar to specimens of 1 week after surgery, and no bone formation around graft material was detected. Graft materials were resorbed gradually(Figure 53, 54, 55 & 56). After 4 weeks, an extensive chronic inflammatory reaction was observed around the graft material. No bone formation was detected except in the marginal region. Graft materials were surrounded by fibrous connective tissue and were absorbed partially. Collagen was absorbed completely and was not detected(Figure 57, 58, 59 & 60). At 8 weeks, bone formation within the defect was not found. Graft materials became smaller by absorption, and were surrounded by fibrous tissue. Weak inflammatory cell infiltration was found around fibrous tissue(Figure 61, 62, 63 & 64).

IV. Discussion

Finding an ideal bone substitute material for grafting has been the goal of researchers for many years. Several bone substitutes have been popularized during the past 10 years and used often by clinicians who deal with ridge augmentation and the reconstruction of osseous defects[1]. The autogenous bone graft is the most predictable material that stimulates non-differentiated mesenchymal cells to form bone cells and also serves as a scaffold for new bone ingrowth[1,3]. However, autogenic bone graft requires a second surgical site with the risk of associated morbidity, the surgeon has limited quantities available.

The use of xenogenic grafting materials derived from bovine bone overcame many of the problems associated with autograft as mentioned above. Several experimental and clinical studies have shown xenogenic grafting materials to be highly biocompatible and osteoconductive[25,26]. Since interests about xenogenic bone have been increasing, a new type of xenogenic grafting materials derived from bovine bone was recently developed, which coated with Ca-P nanocrystal thin film for superior regenerative ability. The present study evaluated the osseous response around Ca-P coated xenogenic bone and compared osteogenic potential of Ca-P coated xenogenic bone to that of combination with type I collagen derived from bovine tendon as a biocompatible binder to prevent migration of the bone particle.

In the present study, we chose to use the adult rabbit calvarium which has many similarities to the maxillofacial region. Morphologically and embry-

ologically, the calvaria develops from a membrane precursor and thus resembles the membranous bone of the face. Anatomically, the calvaria consists of two cortical plates with regions of intervening cancellous bone similar to the mandible[32].

The defects created in the present study were not meant to be critical size defects defined as the smallest size intraosseous wound in a particular bone and species of animal that will not heal spontaneously during the lifetime of the animal. Dodde et al.[33] reported that the critical size of cranial defect in the rabbit model is 1.5cm. But, Jansen et al.[34] reported that the defect created were not meant to be critical size defect, because each rabbit should serve as its own control. So, in the present study, we chose to use the 5mm-diameter calvarial defect, which actually showed complete closure or bone bridging at 2 weeks on nongrafted control group. The purpose for chosing this experimental model was that one calvaria of rabbit should contain 4 defects to compare the effectiveness of graft materials under the same condition, because the healing capacity of individual rabbit was so various in nature.

In the present study, Ca-P xenogenic bone particles showed biocompatible, osteoconductive in initial healing phase, but did not reveal active bone healing. bone was observed in direct contact with the Ca-P coated xenograft at 2 weeks after surgery. Yet, at four weeks after surgery, though some bone formation was evident on the defect, the implants were surrounded by fibrous connective tissue containing macrophage and a few multinucleated giant cells. After 8 weeks, mild chronic inflammation was still present. Newly formed bony trabeculae had invaded the defects from periphery but, the cortical bony plate was still missing. Then, in nongrafted control group, healing was more rapid compared with defects containing Ca-P coated xenograft. After 2 weeks, newly formed trabecular bone almost filled the entire defect. Four weeks after surgery, healing of the cavities was more mature and after 8 weeks a cortical bony plate had formed. Some studies reported that deproteinized bovine bone interfered bone healing at mineralization phase[35] and provoked host inflammatory response[36]. These chronic inflammatory response may inhibit osteoinduction[37-39]. However, other study reported that Ca-P coated xenogenic bone is effective at new bone formation of extraction socket in adult dogs[28]. This result was more effective than our study, the difference could possibly be caused by the healing potential of experimental site. Compared to the calvarial defect, the extraction socket site is preferable due to improved blood supply and immobilization of graft particles.

In the present study, absorption of Ca-P xenogenic bone was initiated after 1 week and sustained at 8 weeks. At 8 weeks after surgery, implant material dispersed in defect and size of material diminished considerably. However, the current observation time (8 weeks) may not be sufficient for deproteinized bovine bone to completely disappear. Some authors have claimed that bovine bone mineral undergoes resorption[36]. Other investigators have claimed that the resorption process of bovine bone mineral is very slow, if it resorbs at all[40-41]. The difference between reports may be explained by a difference in individuals, level of inflammation, characteristic of the surface, the area of defect. Additional long term studies are needed to determine absoption of xenogenic graft derived from bovine bone.

We used the type I collagen as a biocompatible resorbable binder to prevent migration of the particulate bone materials. The collagen is a major protein component of connective tissue. In biomaterial science, collagen is used for suture, soft tissue augmentation[42] and as a skin substitute[43]. Collagen, predominantly type I, constitutes about 90% of the

organic portion of the bone matrix. It has a very low antigenicity, is relatively easy metabolized, and is able to stimulate cell to form new tissue[44,45]. Several investigators on implantation with bone substitutes impregnated with collagen reported profound bony ingrowth with no significant evidence of graft resorption and suggested that collagen may act as a stimulus for new bone formation[46,47]. Other investigators demonstrated that collagen does not alter healing, is possible to use it as a biocompatible resorbable binder to confine particulate ceramic implants and inhibit their migration[48,49].

However, in the present study Ca-P coated xenogenic bone combined type I collagen was no new bone formation surrounding implants in histologically. Compared to the Ca-P coated xenogenic graft alone, the combination with type I collagen showed more severe inflammatory response. The implants were surrounded by fibrous connective tissue containing macrophage and a few multinucleated giant cell. Chronic inflammation with lymphocytes, large amounts of multinucleated giant cells dominated the central parts of the defects. The implant material provoked a foreign body response. The our observations were similar to the findings of Levy et al.[50] They tested the capacity of a calf-skin collagen-mineral gel to induced bone regeneration within surgically-created periodontal defect in one dog and four monkeys. no bone regeneration occured within 8 to 14 weeks. For reasons of this result, ox fibrin and ox collagen of fibrin-collagen paste may elicit immunological reactions that could inhibit osteoinduction[51]. Sela et al.[52] said that low pH resulted from collagen-mediated inflammatory reaction inhibited bone regeneration. Previous studies have indicated that chronic inflammation may inhibit osteoinduction[37-39]. Moreover, bacterial contamination during surgery might be a contributing factor to the lack of osteogenesis in our study.

In the present study, at 2 weeks there was a small quantity of residual collagen, and at 4 weeks no remnant was observed. Blumenthal et al.[53] demonstrated that the at 4-week specimens, the collagen grafting material derived from bovine dermis was present. In 10-week specimens, collagen graft had been replaced by host connective tissue in surgically created defect of dog. Two types of collagen (atelocollagen and tendon collagen) with cross-linking by various processing methods and various extents were implanted into a dissection site within rat palatal gingiva[54]. According to this report, the rate of collagen dissolution were various by sorts, degree of cross-linking, processing method of collagen and response of host. Also they demonstrated dissolution of non cross-linked tendon collagen that is similar to our experimental collagen. In 14 day specimens, collagen graft were completely absent. This result is shorter than in the present study, the difference could possibly be caused by experimental animals.

We used the autogenous bone particles as positive control to compare other groups. In this study, the autografts also showed a higher incidence of union between the opposed bony surface when compared with the other groups and had both osteoconductive and osteoinductive properties. This observation was similar to the finding of the previous study[55].

In conclusion, implantation of Ca-P coated xenogenic bone in preformed bone defects in rabbits was biocompatible and osteoconductive in the initial healing phase, but it did not stimulate bone healing. Graft particle were separated from host bone by a well-defined connective tissue capsule. The addition of collagen to Ca-P coated xenograft changed the tissue response. Collagen interfered with the healing of bony defects filled with Ca-P coated xenograft, whereas autogenous bone

implantation was observed significant bone regeneration and exhibited an advanced remodelling of the lamellar new bone structure. Therefore, further investigations are required to systematically study the development of grafting materials with osteoconductive and osteoinductive properties and development of biocompatible resorbable binder without inflammatory response.

V. Summary

The purpose of this present study evaluated the osseous response around Ca-P coated xenogenic bone and compared osteogenic potential of Ca-P coated xenogenic bone to that of combination with type I collagen derived from bovine tendon as a biocompatible binder to prevent migration of bone particle on the repair of calvarial defects in rabbits.

To study the effects of Ca-P coated xenogenic bone and collagen on bone healing, four 5-mm-diameter skull defect were made in calvaria with trephine filled with an autogenous bone chip or Ca-P coated xenogenic bone or Ca-P coated xenogenic bone and type I collagen (1:1 mixture by volume) or left empty. The defects were evaluated histologically at 1, 2, 4 and 8 weeks following implantation.

Ca-P coated xenogenic bone at the calvarial defects of rabbits showed osteoconductivity at the margin of defect in the early stage of bony healing, but no direct contact with new bone was observed. With time passed by, it was resorbed slowly and showed consistent inflammatory reaction. An additional use of type I collagen derived from bovine tendon improved clinical handling, but no new bone formation was observed histologically. Above all, autogenous bone graft showed most prominent healing in quantity and density of new bone formation.

According to this study, the use of Ca-P coated xenogenic bone alone and combination with type I collagen did not showed effective healing in quantity and density of new bone formation.

VI. References

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사진부도 설명

Figure 1, 2, 3, 4. Non-grafted control defects (group 1, 1 week).
Photomicrograph shows mild infiltration of inflammatory cells. Limited immature new bone trabeculae(NB) are found at the margin of the defects. (Figure. 3 & 4. Masson's Trichrome, ×20, ×100), (Figure 5 & 6, H-E, ×20, ×100)

Figure 5, 6, 7, 8. Non-grafted control defects (group 1, 2 weeks).
Photomicrograph shows mild infiltration of inflammatory cells. Newly formed bone(NB) completely bridges the previous defect but, the regenerated bone has not attained a thickness similar to that of the surrounding bone. (Figure. 7 & 8. Masson's Trichrome, ×20, ×100), (Figure 9 & 10, H-E, ×20, ×100)

Figure 9, 10, 11, 12. Non-grafted control defects (Group 1, 4 weeks).
Photomicrograph shows no infiltration of inflammatory cells, and newly formed bone completely bridges the previous defect. (Figure. 11 & 12. Masson's Trichrome, ×20, ×100), (Figure 13 & 14, H-E, ×20, ×100)

Figure 13, 14, 15, 16. Non-grafted control defects (Group 1, 8 weeks).
Photomicrograph shows no infiltration of inflammatory cells and mature new bone with marrow space. (Figure. 15 & 16. Masson's Trichrome, ×20, ×100), (Figure 17 & 18, H-E, ×20, ×100)

Figure 17, 18, 19, 20. Defects filled with autogenous bone chip (Group 2, 1 week)
Photomicorgraph shows mild infiltration of inflammatory cells. At surrounding the remaining bone graft particle(*) as well as at the margin of bone defect, formation of immature bone trabeculae was observed. (Figure. 19 & 20. Masson's Trichrome, ×20, ×100), (Figure 21 & 22, H-E, ×20, ×100)

Figure 21, 22, 23, 24. Defects filled with autogenous bone chip
(Group 2, 2 weeks)
Photomicrograph shows no infiltration of inflammatory cells. Regenerated bone has attained a thickness similar to that of surrounding bone and is seen in direct apposition to the autogeneous bone particle(*). (Figure. 23 & 24. Masson's Trichrome, ×20, ×100), (Figure 25 & 26, H-E, ×20, ×100)

Figure 25, 26, 27, 28. Defects filled with autogenous bone chip
(Group 2, 4 weeks)
Photomicrograph shows no infiltration of inflammatory cells. Grafted bone was in union with new bone without intervention of soft tissue. (Figure. 27 & 28. Masson's Trichrome, ×20, ×100), (Figure 29 & 30, H-E, ×20, ×100)

Figure 29, 30, 31, 32. Defects filled with autogenous bone chip
(Group 2, 8 weeks)
Photomicrograph shows no infiltration of inflammatory cells. Defects were healed mature bone with homogeneous thickness, thick and dense sponge bone. (Figure. 31 & 32. Masson's Trichrome, ×20, ×100), (Figure 33 & 34, H-E, ×20, ×100)

Figure 33, 34, 35, 36. Defects filled with Ca-P coated resorbable natural bone mineral (Group 3, 1 week)
Photomicrograph shows moderate infiltration of inflammatory cells. New bone formation was found at the margin of the defects. Partially, it was in union with graft material(⁂) without intervention of soft tissue. (Figure. 39 & 40. Masson's Trichrome, ×20, ×100), (Figure 41 & 42, H-E, ×20, ×100)

Figure 37, 38, 39, 40. Defects filled with Ca-P coated resorbable natural mineral (Group 3, 2 weeks)
Photomicrograph shows a moderate partially inflammatory response. Newly formed bone(↑) was seen in direct apposition to the bone particle(⁂). Bone graft was resorbed by multinucleated giant cells in some parts. (Figure. 43 & 44. Masson's Trichrome, ×20, ×100), (Figure. 45 & 46, H-E, ×20, ×100)

Figure 41, 42, 43, 44. Defects filled with Ca-P coated resorbable natural bone mineral (Group 3, 4 weeks)
Photomicrograph shows chronic inflammatory response. Graft materials(⁂) were surrounded by fibrous connective tissue and was partially absorbed. (Figure. 47 & 48. Masson's Trichrome, ×20, ×100), (Figure. 49 & 50, H-E, ×20, ×100)

Figure 45, 46, 47, 48. Defects filled with Ca-P coated resorbable natural bone mineral (Group 3, 8 weeks)
Photomicrograph shows chronic inflammatory response. New bone formation(NB) was seen mainly around periosteum. No direct union with graft material(⁂) was detected. (Figure. 51 & 52. Masson's Trichrome, ×20, ×100), (Figure. 53 & 54, H-E, ×20, ×100)

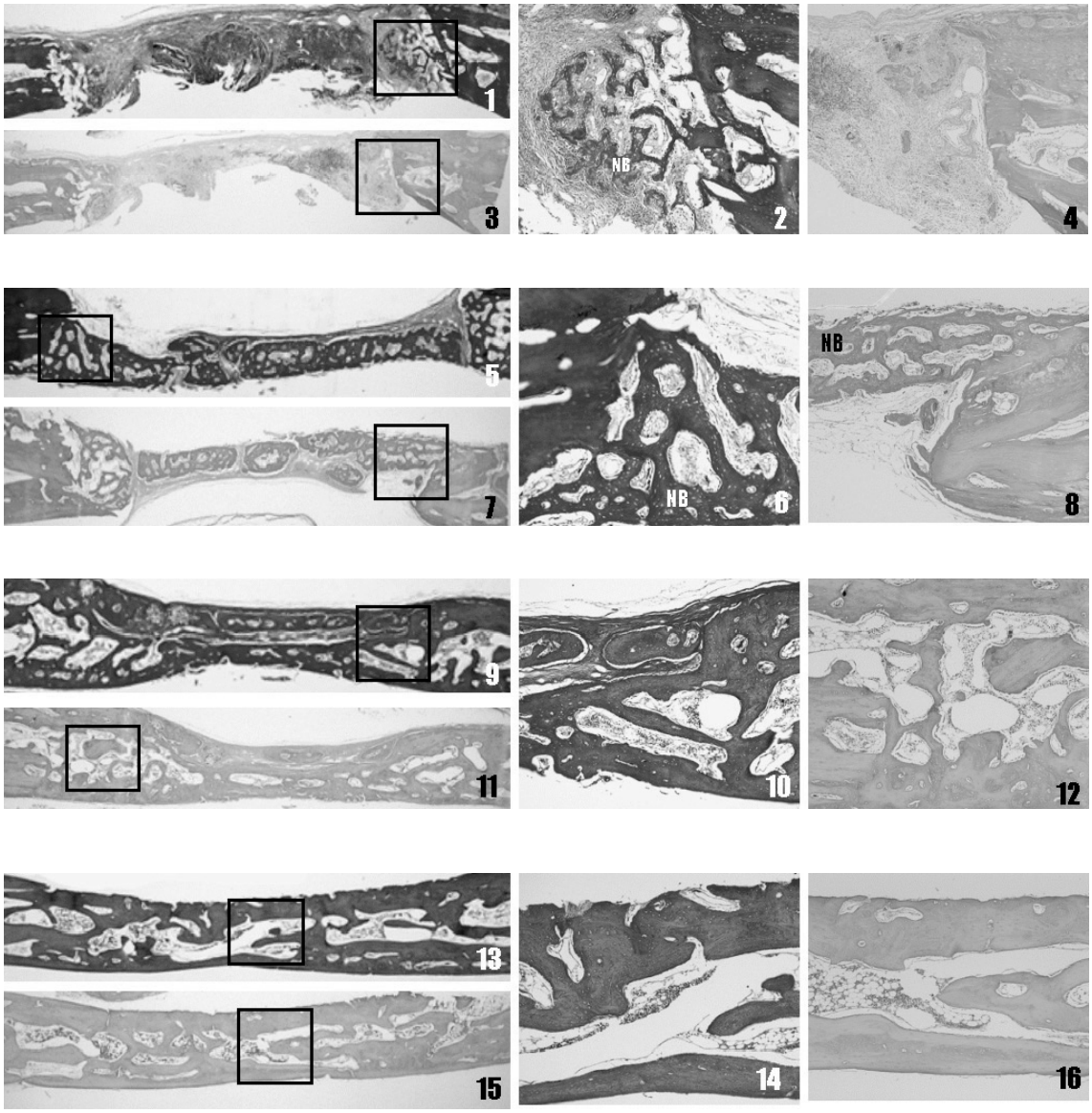
Figure 49, 50, 51, 52. Defects filled with Ca-P coated resorbable natural bone mineral and type I collagen (1:1 mixture by volume)
(Group 4, 1 week)
Photomicrograph shows a marked infiltration of inflammatory cells. New bone formation around established bone was seen, but not around xenograft(⁂) and collagen(▲). (Figure. 55 & 56. Masson's Trichrome, ×20, ×100), (Figure. 57 & 58, H-E, ×20, ×100)

Figure 53, 54, 55, 56. Defects filled with Ca-P coated resorbable natural bone mineral and type I collagen (1:1 mixture by volume) (Group 4, 2 weeks)
Photomicrograph shows reduced infiltration of inflammatory cells. No bone formation around graft material(⁂) was detected. Graft materials were resorbed gradually. (Figure. 59 & 60. Masson's Trichrome, ×20, ×100), (Figure. 61 & 62, H-E, ×20, ×100)

Figure 57, 58, 59, 60. Defects filled with Ca-P coated resorbable natural bone mineral and type I collagen (1:1 mixture by volume) (Group 4, 4 weeks)
Photomicrograph shows An extensive mononuclear inflammatory reaction. No bone formation was detected except in the marginal region. Graft materials(**) were surrounded by fibrous connective tissue and was partially absorbed. (Figure. 63 & 64. Masson's Trichrome, ×20, ×100), (Figure. 65 & 66, H-E, ×20, ×100)

Figure 61, 62, 63, 64. Defects filled with Ca-P coated resorbable natural bone mineral and type I collagen (1:1 mixture by volume)(Group 4, 8 weeks)
Photomicrograph shows a weak inflammatory cell infiltration. Graft material(**) became smaller by absorption, and was surrounded by fibrous tissue. (Figure. 67 & 68. Masson's

사진부도 (I)



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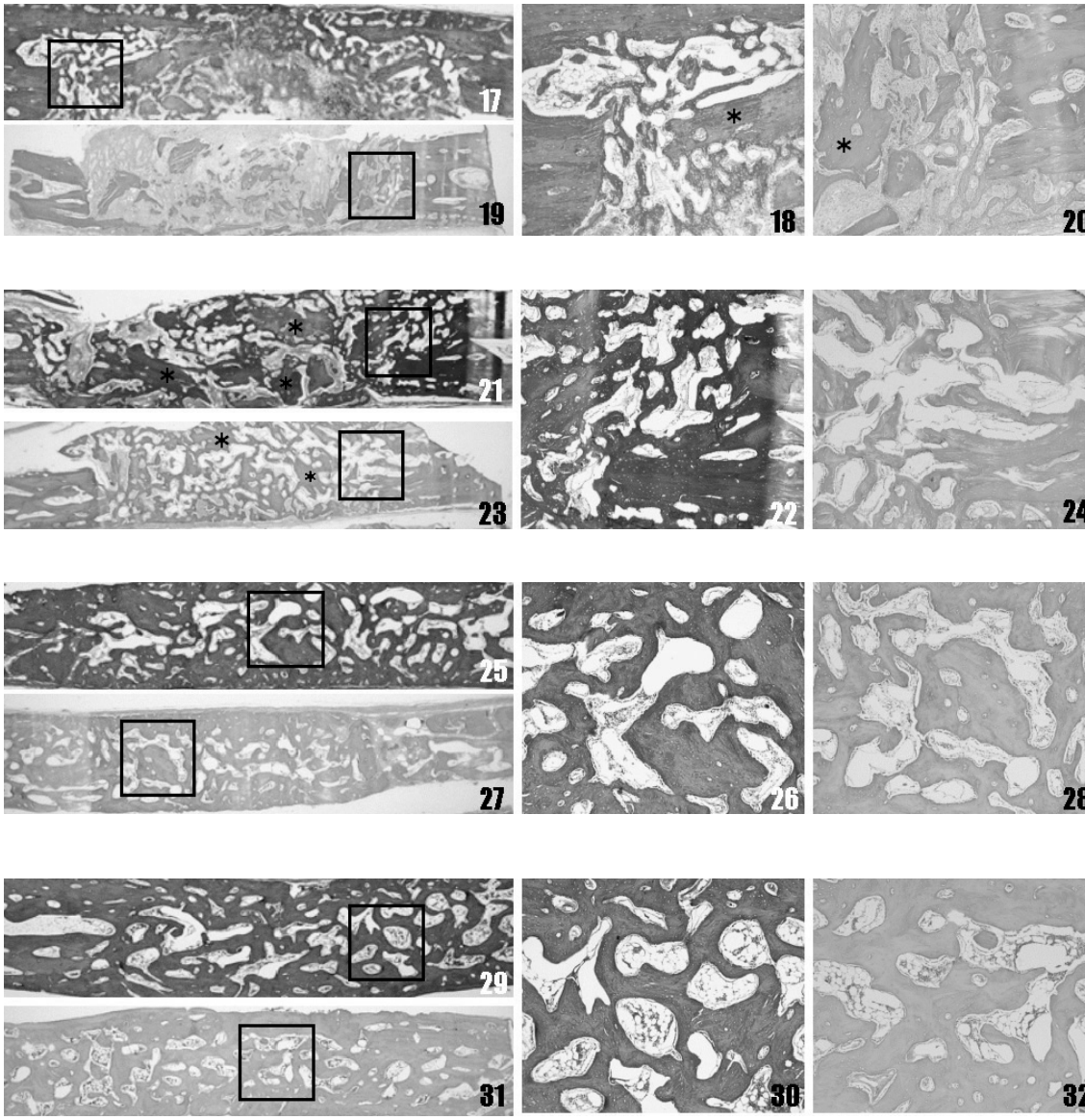

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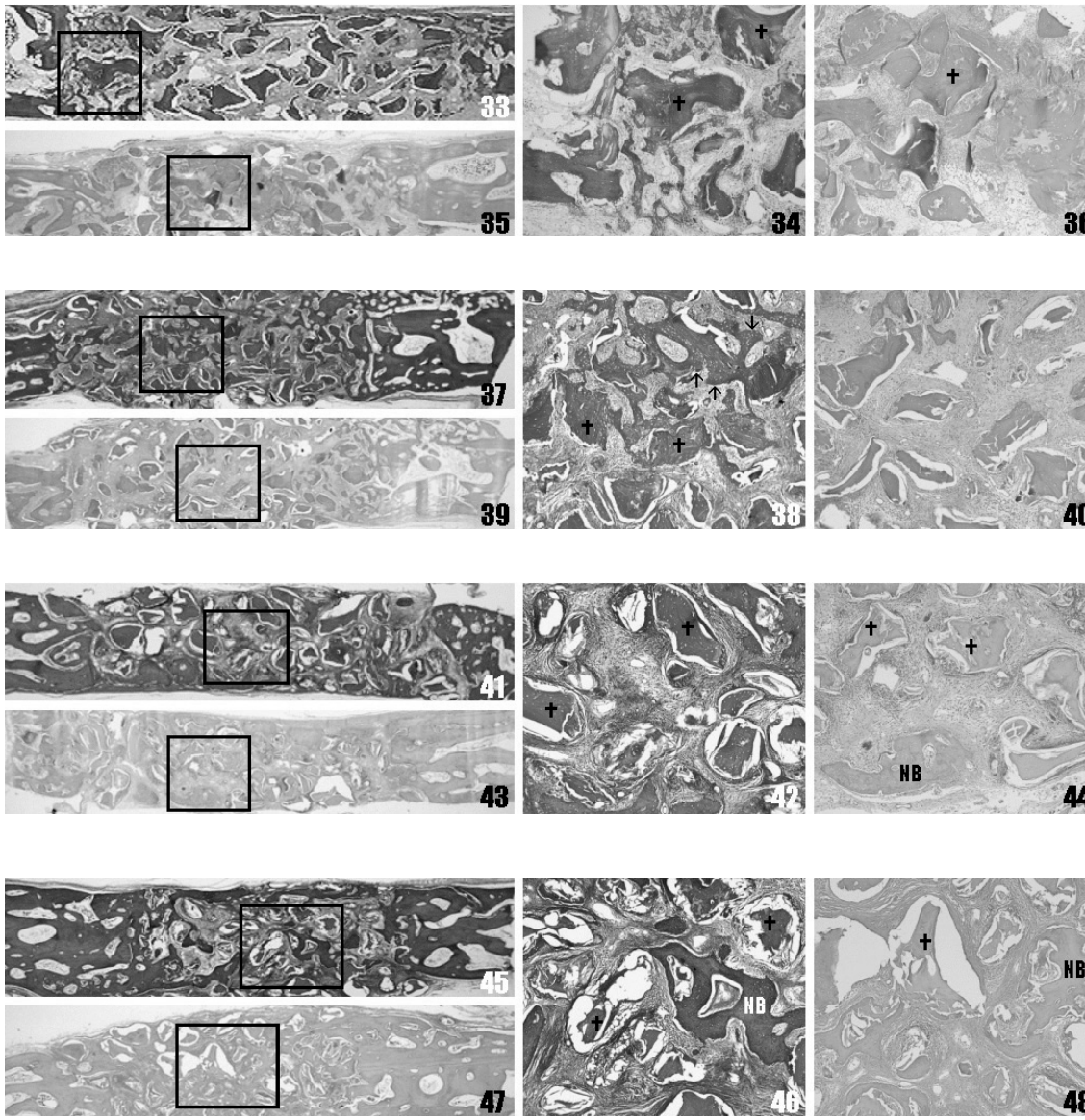
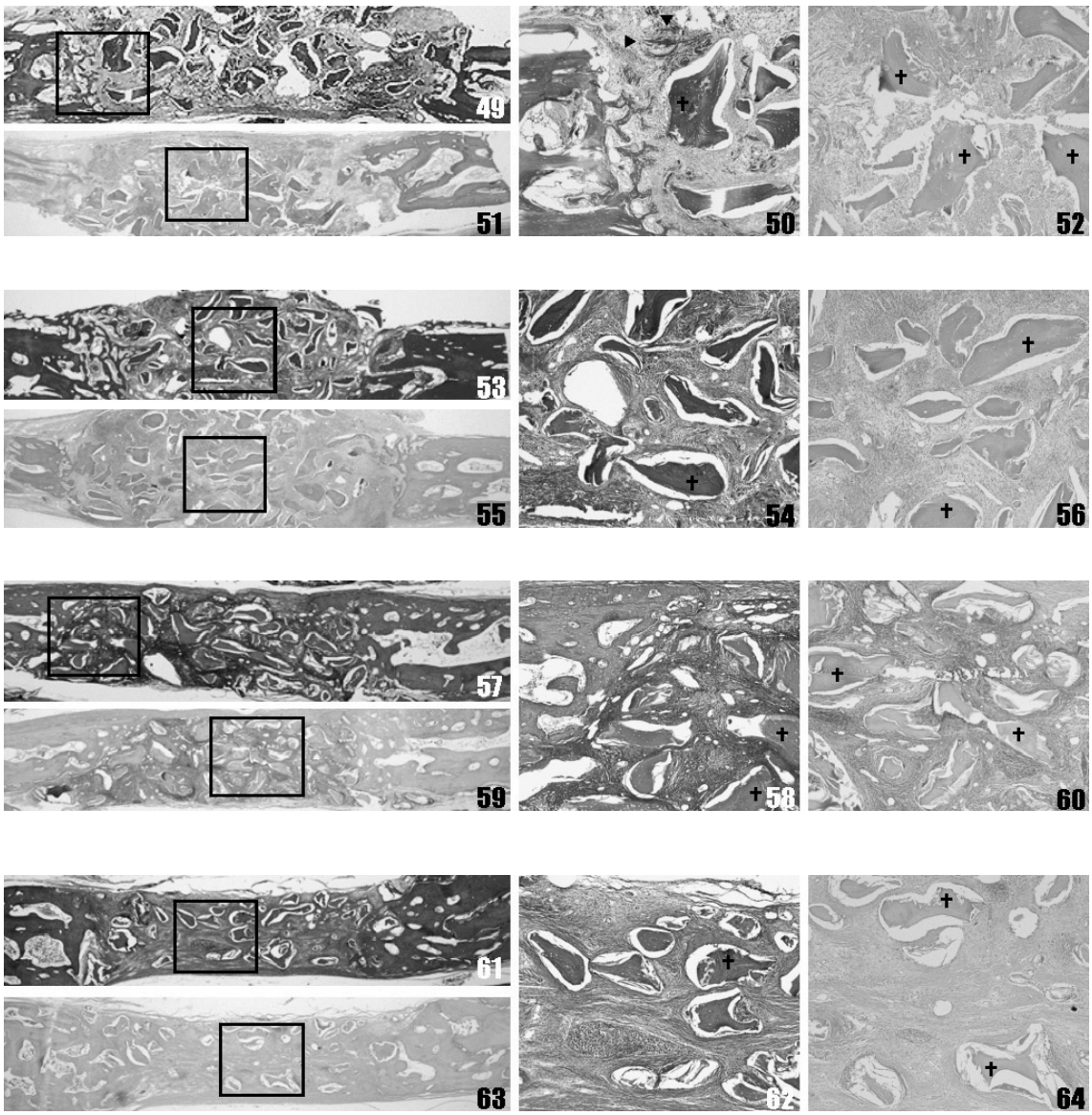


사진부도 (IV)



-국문초록-

Ca-P 박막 이종골과 제 1형 교원질이 토끼 두개골 결손부의 골재생에 미치는 영향

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골재생을 위해 사용되는 골이식재로 자가골, 동종골, 이종골 등이 있다. 자가골은 가장 예지성이 높은 골이식재 이지만, 부가적인 수술, 환자의 동통과 불편, 채취하는 양의 제한, 비용의 증가 등의 단점이 있다. 따라서 많은 연구자들은 오랫동안 자가골을 대체할 골이식재 개발에 힘써왔고, 다양한 연구가 있었다. 소로부터 유래한 이종골은 천연 다공성의 골 무기질로서, 인간의 골의 구조와 유사하면서, 골 전도성이 있고, 생체 적합성이 뛰어나다고 보고되었다. 이에 최근에 개발된 Ca-P 박막 이종골과 조작성을 용이하게 하기 위해 부가적으로 type I collagen을 혼합한 골이식재를 토끼 두개골 결손부에 매식하여 골형성 능력 및 주변 조직의 반응을 보고자 하였다.

총 16마리의 New Zealand white rabbits를 사용하였고, 두개골에 4부위의 결손부를 형성한 후, 다음과 같이 적용하였다. 이식재를 넣지 않은 군을 음성대조군으로, 자가골 분말을 이식한 군을 양성대조군으로, Ca-P 박막 탈단백 우골 분말을 이식한 군을 실험1군으로, Ca-P 박막 탈단백 우골 분말과 type I collagen을 같은 부피로 혼합하여 이식한 군을 실험2군으로 하였다. 1, 2, 4, 8주째 4마리씩 희생하여, H-E 염색과 Masson's trichrome 염색을 시행한 후, 광학현미경을 사용하여 조직학적으로 관찰하였다.

토끼 두개골 결손부에 이식한 Ca-P 박막 탈단백 우골은 골성회복초기에 골결손부 변연에서 골전도성을 보였지만, 완전한 골성회복을 이루지 못하였고, 신생골과 직접적인 유합을 보이지 않았다. 또, collagen의 부가적인 사용은 조작성은 가장 우수했으나, 조직소견상 신생골의 형성을 보이지는 않았다. 반면 자가골을 이식한 부위는 신생골 형성양과 밀도에 있어서 가장 우수한 결과를 보였다.

Key words; Xenogenic bone, Type I collagen, Calcium-phosphate