pISSN 1598-298X / eISSN 2384-0749
J Vet Clin 32(5) : 410-416 (2015)
http://dx.doi.org/10.17555/jvc.2015.10.32.5.410

Journal of Veterinary Clinics
www.ksvc.or.kr

Evaluation of the Bone Defect Regeneration after Implantation with Cuttlebone in Rabbit

Sangcheol Won, Joo-Myoung Lee, Hyunjung Park, Jongpil Seo and Jongtae Cheong[1]

College of Veterinary Medicine · Veterinary Medical Research Institute, Jeju National University, Jeju 63243, Korea

(Accepted: October 23, 2015)

Abstract : Bone grafting is widely used to bridge major bone defects or to promote bone union. In the evaluation of bone defect regeneration, 5 mm-diameter defects were created in rabbit calvaria. Concerning biocompatibility, fibrous capsule thickness of CBHA (hydroxyapatite from cuttlebone) was significantly thinner than that of CB (cuttlebone) and CHA (hydroxyapatite from coral) ($p < 0.05$) at 2 and 4 weeks after implantation. Concerning 12-week total changes of radiologic gray-level histogram, CBHA was significantly higher than CHA ($p < 0.05$). In the evaluation of bone defect regeneration, bone formation of CHA was significantly higher than that of CB and CBHA ($p < 0.05$). Based on the clinical and histological results, CBHA would be a safe material for use inside the body and has more effective osteoconduction than CB. It is suggested that CBHA is a valuable bone graft material.

Key words **:** Bone graft, cuttlebone, hydroxyapatite, coral, scaffold.

Introduction

Natural calcium carbonate (CC; $CaCO_3$) such as coral, eggshell has been used as a bone substitute material (5,6,7,10,19, 26). Through a hydrothermal reaction, the $CaCO_3$ skeleton of CC is changed to hydroxyapatite (HA: $Ca_{10}(PO_4)_6(OH)_2$). HA has been isolated from coral (CHA) (24), eggshell (23), and cuttlebone (CBHA) (12,18). The basic difference between HA and CC is that CC is biodegradable and HA is not or only very little (26). Porous HA is biocompatible and osteoinductive (3). HA has attracted a great deal of interest as a biomaterial for implants and bone augmentation since its chemical composition is close to that of bone (18). Cuttlebone (CB), which is composed of CC has a porous structure with all pore interconnected throughout the skeleton and CB.

The surface area of 12 other implants was compared and reported that the surface area of demineralized bovine bone was 79.7 m^2/g. This was five times higher than the surface area of other bone regeneration materials (27). But, the surface area of CB (386 m^2/g) was four times higher than that of demineralized bovine bone. Buffering capacity of CB showed the same pattern as that of CC. CB is composed of 53.25% Ca, 26.78% O, 14.90% Na, 4.37% Cl, 0.50% Sr, 0.06% P, 0.06% S, and 0.05% Si (16). Most of calcium in CB was present in a form of CC. Highly interconnective porosities serve as channels for internal growth of blood vessels and increase bone (27) and bone containing macro- and micropores, which are interconnected to allow necessary body nutrients and fluids to be transported, making bone an extremely complex structure (1). Scanning electron microscopy has revealed the multiplier structure of CB (2,16). The minimum pore diameter required for bone ingrowth and angiogenesis into a scaffold is generally considered to be 100 μm (21,22). The porous structure diameter of CB is usually between 200 and 600 μm (2), which is similar to cancellous bone (9). The optimal osteoconductive pore size for ceramics appears to be between 150 and 500 μm (8). CB represent a new xenograft material in cancellous bone (18). Until now, there have been no reports on the potency of CB as a xenograft bone substitute in compact bone studies.

The purpose of this study was to compare bone defect regeneration after implantation of CB in a rabbit calvarial defects model. Bone defect regeneration was measured by radiologic, histologic, and histomorphometric methods.

Materials and Methods

Fabrication of implants

The CB1 (Cuttlebone after defatting and freeze-drying), CB2 (Cuttlebone after removing organic components) and CBHA (Hydroxyapatite from CB2) were prepared from the same genus of cuttle bone (*Sepia esculenta*). The CHA implants used consisted of HA from coral (BoneMedik®, Metabiomed, Korea). The CB1 implants were processed as follows; for defatting, cuttle bone was immersed in chloroform and methanol (1:1) for 6 days. Defatted cuttle bone was frozen at −80ºC for 24 hours. And then frozen cuttle bone was freeze-dried at −80ºC for 72 hours. And freeze-dried cuttle bone was sterilized by ethylene oxide for 24 hours (4). The CB2 implants were processed as follows; for removing of the organic components the cuttle bone was immersed in 4% sodium hypochlorite solution for 24 hours and washed with distilled water for 24 hours. The cuttle bone was dried for 12 hours at 70ºC in dry oven, and sterilized by ethylene oxide for 24 hours (18).

[1]Corresponding author.
E-mail : cjt123@jejunu.ac.kr

CBHA implants were processed in hydrothermal synthesis: CB2 was put in 2M $(NH_4)_2HPO_4$ in a Teflon® lined hydrothermal bomb (Hydrothermal Reactor System®, Hanwoul Engineering, Korea) and heated for 16 h at 180°C. Then, the block was immersed in 2M $(NH_4)_2HPO_4$ and treated at 200°C for 24 h hydrothermally. After thoroughly washing with distilled water, the block was dried at 90°C (18). After X-ray diffraction (XRD) examination of the block, it was used as CBHA. These implants were shaped into cylindrical disks about 5 mm in diameter and 2 mm in thickness and were sterilized by ethylene oxide gas.

CB1bmp implant was CB1 with rhBMP-2 (Recombinant human bone morphogenetic protein 2). The rhBMP-2 (rhBMP2®, Korea Bone Bank, Korea) was produced by a Chinese hamster ovarian cell expression system to yield a glycosylated 26 kilodalton homodimer. rhBMP-2 (100 μg/cm^3) was instilled into CB1 with use of a syringe (fine needle infiltration) at the time of the operation (13).

Experimental animals and surgical procedure

Twenty seven 9-month-old, 3.2 ± 0.5 kg male New Zealand white rabbits were kept under a standard light-dark schedule and were fed a stock diet and had access to tap water *ad libitum*. The experimental protocol was approved by the Animal Care and Use Committee, Jeju National University (approval number 2010-0042). The rabbits were divided into six groups as shown in Table 1. Aseptic surgical technique was applied during the surgical procedure. They were anesthetized with an intramuscular injection of a dose of tiletamine/zolazepam (Zoletil50®, Virbac, France). After the anesthesia, incision sites were shaved. The incision sites were then washed with 70% ethanol and scrubbed with 10% povidone iodine. Incision sites were exposed with a sagittal incision through the skin and the periosteum at the midline of the calvaria. With the use of a medical trephine burr (TPHB-B5®, Osung Mnd, Korea), four standardized full-thickness 5 mm-diameter osseous defects were created in the calvarium of each rabbit under saline irrigation. In group CB1, CB1bmp, and CHA, the left rostral defect was filled with CB1 disk, the left caudal defect was filled with CB1bmp disk, the right caudal defect was filled with CHA disk, and the right rostral defect was kept and used as a negative control.

In groups CB2 and CBHA, both sides of the rostral and caudal defects were filled with CB2 and CBHA disks. The skin was closed with 3-0 nylon. Immediately following implantation, the rabbits were injected subcutaneously with a dose of penicillin G procaine (Combimycin Inj.®, Green Cross Veterinary Products, Korea) for 3 days.

Table 1. Experimental design for the bone defect regeneration in rabbits

Rabbits	Group	Application	
		Site	Types of implants
n = 18	Blank	R. Ros. Calvaria	Blank
	CHA	R. Cau. Calvaria	CHA
	CB1	L. Ros. Calvaria	CB1
	CB1bmp	L. Cau. Calvaria	CB1 + rhBMP-2
n = 9	CB2	R. Ros. Calvaria	Cuttlebone 2
		L. Ros. Calvaria	Cuttlebone 2
	CBHA	R. Cau. Calvaria	HA from cuttlebone
		L. Cau. Calvaria	HA from cuttlebone

L: Left, R: Right, Ros: Rostral, Cau: Caudal, n: number of experimental animal, CB: Cuttlebone, CHA: Hydroxyapatite from coral, rhBMP-2: Recombinant human bone morphogenetic protein-2

Radiographic interpretation and radiologic gray-level histogram (brightness) evaluation

Nine rabbits were euthanized at 4, 8, and 12 weeks postoperatively. The implants and the surrounding calvarial bone were removed. The defect regions was submitted to contact radiography in a dorso-ventral plane with a Kodak Direct View CR975 X-ray System (Eastman Kodak, USA) at an exposure of 42 kVp and 6.4 mAs. Bone formation was evaluated by gray-level histogram in radiograph processing using Adobe® Photoshop® 7.0 (Adobe Systems, USA). The histogram of each implant was measured before implantation to calibrate the radiologic gray-level histogram. Then, the histogram of the implants were measured at 4, 8, and 12 weeks after implantation, and the changes of the histogram were recorded on the basis of a calibrated histogram.

Histologic investigations and histomorphometric analysis

Removed calvarial bone was immersed in 10% phosphate-buffered formalin for 3 days and decalcified for 7 days using 5% formic acid. It was dehydrated in an ethanol series, embedded in paraffin, and cut into 6 mm-thick sections. The sections were stained with H&E stain. The defect closure was determined with a CCD camera-based digital image analysis system (Fig 1). The rate of bone defect regeneration was determined by a histomorphometric method: rate of bone defect regeneration (%) = [(original defect width, mm) − (remained defect width, mm)] / (original defect width, mm) × 100 (14,20,25).

Statistical analysis

The Statistical Package for the Social Science, version 17.0 software (SPSS, USA) was used for data analysis. Mann-

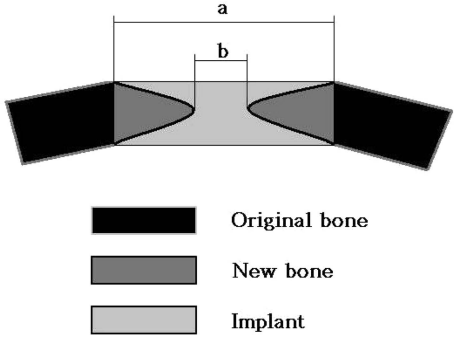

Fig 1. Measurement of bone defect regeneration in rabbit calvaria by histomorphometric parameters. The measurement was performed 4, 8, and 12 weeks after implantation.

Whitney's *u*-test was used to evaluate differences between each group. A p value ≤ 0.05 was considered statistically significant.

Results

Radiologic evaluation

At 4 weeks, in group CB1 and CB1bmp, the radiopaque implant sites were observed. In group CHA, the implant site was more radiopaque than the sites in group CB1 and CB1bmp. In group CB2, partial bone regeneration was observed and the radiopaque implant site was delineated from the surrounding bone by a radiolucent border. In group CBHA, bone regeneration processing towards the centre of the defect was observed and radiopaque implant site was delineated from the surrounding bone by a radiolucent border.

At 8 weeks, the radiopaque implant site in group CB1 revealed a more enlarged circle than group CB1bmp and CHA. In group CB1bmp, a radiopaque implant site was observed and the radiopaque implant site was delineated from the surrounding bone by a radiolucent border. In group CHA, the more radiopaque implant site was observed than group CB1 and CB1bmp. In group CB2, partial bone regeneration was observed and the more enlarged circle was observed than group CBHA. In group CBHA, bone regeneration processing towards the centre of the defect was observed and the radiopaque implant site was delineated partially from the surrounding bone by a radiolucent border.

At 12 weeks, in group CB1 and CB1bmp, partially radiopaque implant sites were observed. In group CHA, a more radiopaque implant site was observed than in groups CB1 and CB1bmp. In group CB2, a radiopaque implant site was observed and the circle was more enlarged than in group CBHA. In group CBHA, bone regeneration processing towards the centre of the defects was observed. At 12 weeks, bone regeneration strikingly advanced in group CBHA comparing with the group CB1, CB2, and CB1bmp.

Total changes of radiologic gray-level histogram (brightness)

In group CBHA, total changes of the radiologic gray-level histogram was significantly higher than that of the group CHA during 12 weeks after implantation ($p < 0.05$), but there was no significant difference compared with that of the group CB1, CB2, and CB1bmp. In group CHA, total changes of radiologic gray-level histogram during 12 weeks after implantation was significantly lower than that of the other groups ($p < 0.05$). In group CB1, CB2, CB1bmp, and CBHA, total changes of the radiologic gray-level histograms were similarly changed during the 12 weeks, but there was no significant difference compared with that of the other groups. At 12 weeks, group CHA was significantly lower than that of the other group ($p < 0.05$) (Fig 2). This evaluation was calibrated as the numerical value.

Histomorphometric analysis for bone defect regeneration

The rate of bone defect regeneration of the implanted site in group CHA was significantly higher than that of other groups at 4 and 8 weeks after implantation ($p < 0.05$). At 12 weeks, the rate of bone defect regeneration in group CHA was significantly higher than that of group CB1, CB2, and CB1bmp ($p < 0.05$). But, there were no significant differences between group CHA and CBHA (Fig 3).

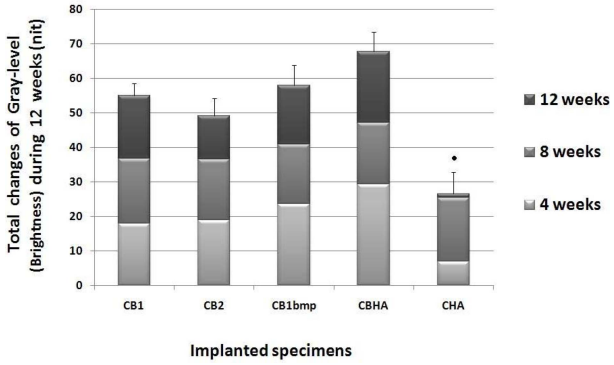

Fig 2. Total 12-week changes of radiologic gray-level histogram in each specimen. The change of gray-level histogram in group CBHA was the highest after implantation, but there were no significant differences between group CB1 and CB1bmp. Values expressed as mean ± SE.
● Significantly lower than other groups after implantation ($p < 0.05$).

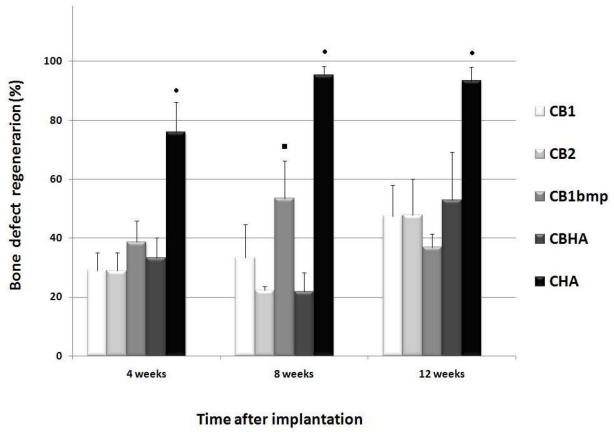

Fig 3. The rate of bone defect regeneration group CB1, CB2, CB1bmp, CBHA, and CHA specimens. Values are expressed as mean ± SE.
● Significantly higher than other groups at 4, 8, and 12 weeks after implantation ($p < 0.05$).
■ Significantly higher than group CBHA at 8 weeks after implantation ($p < 0.05$).

Histologic evaluation

At 4 weeks after implantation, all graft materials in the calvarial defect area were surrounded by moderate to severe dense fibrous collagenous fibers (Fig 4). Multifocal to diffuse inflammation composed of neutrophils, macrophages, and foreign body giant cells were scattered in the adjacent area of fibrous tissues. In the CHA group, proliferated collagenous fibers were invaginated into the graft materials.

At 8 weeks after implantation, overall fibrous stromal reaction in each group was gradually increased, as compared to 4 weeks. However, the inflammatory reaction was dramati-

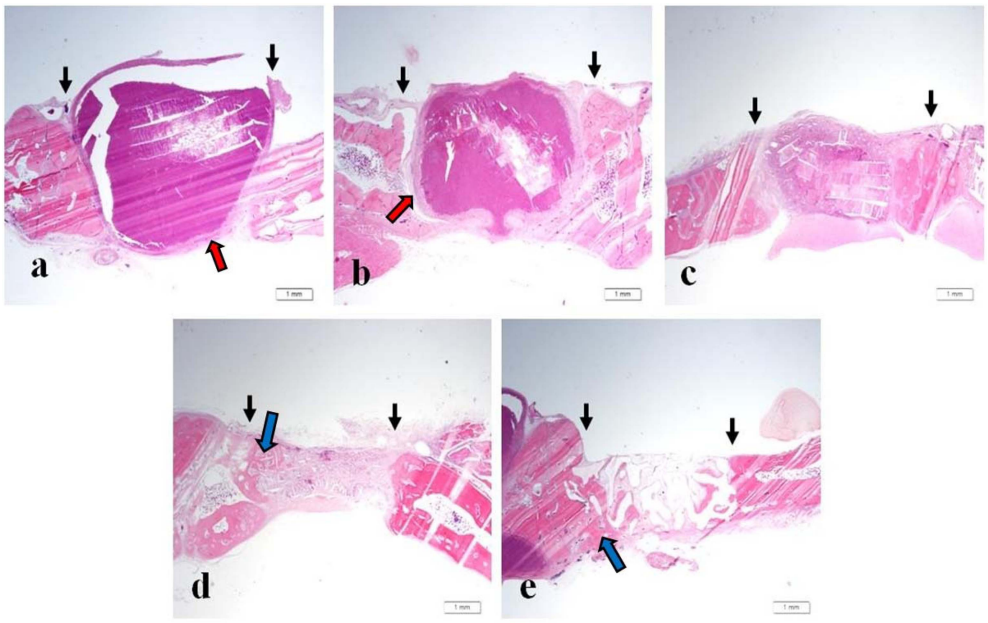

Fig 4. Histologic sections of group CB1 (a), CB1bmp (b), CB2 (c), CBHA (d), and CHA (e) at 4 weeks after implantation (black arrows: defect margins). Group CB1 and CB1bmp were encapsulated by fibrous connective tissue (a, b: red arrows). Bone defect regeneration was observed at defect margins in group CBHA and CHA (d, e: blue arrows). The sections were stained using H&E.

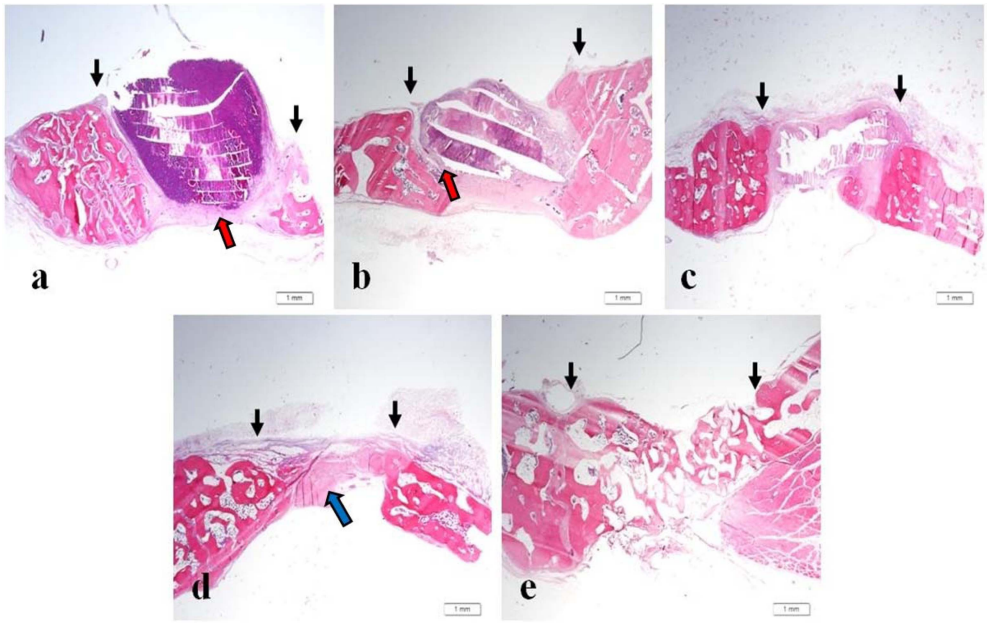

Fig 5. Histologic sections of group CB1 (a), CB1bmp (b), CB2 (c), CBHA (d), and CHA (e) at 12 weeks after implantation (black arrows: defect margins). CB1 and CB1bmp implants were still encapsulated by thick fibrous connective tissue (a, b: red arrows). New bone formation was observed in group CBHA (d: blue arrow). The sections were stained using H&E.

cally decreased. Compared with other groups, most graft materials were replaced by inward growing new bone in group CHA.

At 12 weeks after implantation, variable extents of bone growth into defect areas were observed in all experimental groups (Fig 5). New bone formation extended into graft materials in group CBHA and CHA (Fig 6). However, fibrous stromal and inflammatory reactions still remained in groups CB1, CB1bmp, CB2, but little remained in groups CBHA and CHA. In group CHA, an obvious continuity of cortical bone from the calvaria was observed in both sides of the bone defect. A marked increased number of osteoblasts were present at the surface of bonny spicules. Multifocal bone marrow formation also existed in cortical bone (Fig 5, 6).

Discussion

The critical size defect was not suggested in a rabbit calvarial model (25). The critical size defect depends on the presence of the periosteum (11). A defect with a diameter of

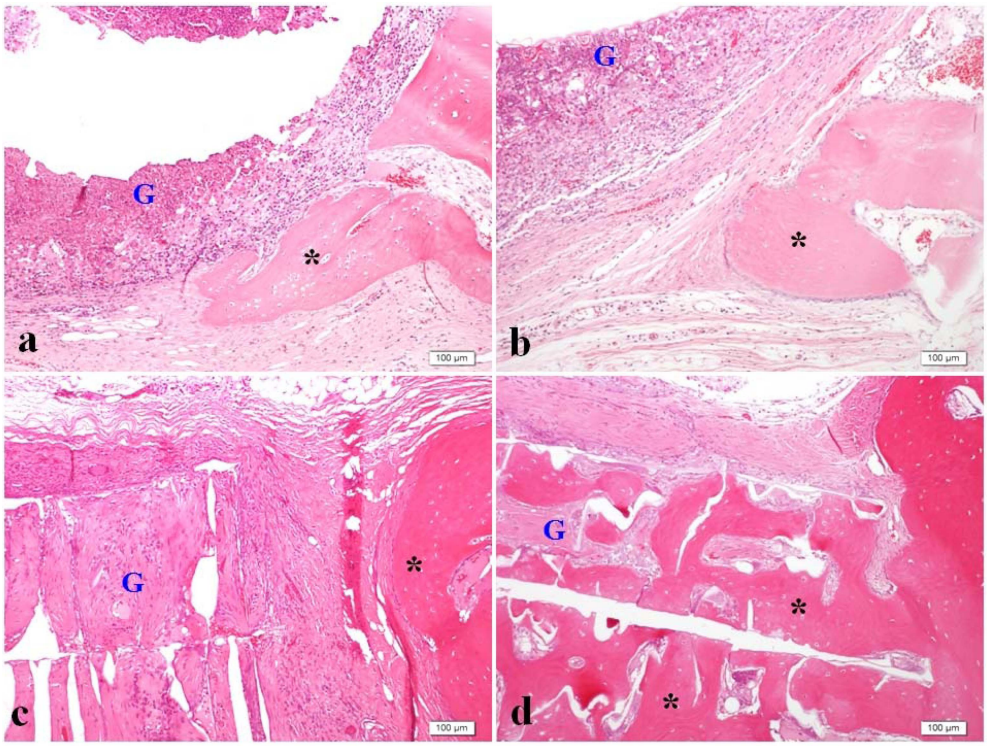

Fig 6. Histologic features of new bone formation in group CB1 (a), CB1bmp (b), CB2 (c), and CBHA (d) at 12 weeks after implantation. New bone formation (asterisks) was observed around the graft material (G) in group CB1 or CB1bmp and inside of graft material in group CBHA. The sections were stained using H&E.

5 mm is a commonly used size a rat models (6,19). Presently, considering the convenience of operation, defects with a diameter of 5 mm were created in the calvaria of rabbits and the periosteum surrounding the implant was widely-removed.

Interconnecting pores are the pathway for new vessels, which is an advantage for new bone formation (22). Blood supply to the cancellous bone is much better than to the compact bone. For this reason, bone regeneration in cancellous bone is faster than in compact bone. Nevertheless, this study was performed to the compact bone, in order to directly observe the bone defect regeneration without cartilage formation.

The lowest dose of rhBMP-2 needed for defect healing was between 40 and 160 μg/cm^3 and suggested a 100 μg/cm^3 dose, based on results in canine ulnar defect model. In this study, the dose of rhBMP-2 was 100 μg/cm^3 (13). From the results, we decided that the dose of rhBMP-2 in CB was suitable or not, because this study was not focused on the determination of dose of rhBMP-2. But the dose of 100 μg/cm^3 was effective in bone defect regeneration.

In bone defect regeneration, radiologic analysis is commonly used to compare the feasibility of bone graft use in different studies (6,7,13,15). In group CHA, a more radiopaque implant site was observed than apparent in the surrounding bone and the other groups at 12 weeks after implantation. Bone regeneration was determined by changes of radiodensity. For this reason, bone regeneration processing of CHA implant could not be observed during 12 weeks after implantation. But, 8 and 12 weeks after implantation, an ill-defined change was apparent between bone and implant, indicating that bone remodeling occurred in the CHA group. This finding is same result with other studies (5,7). In group CB1, the size of defect was enlarged at 8 weeks after implantation.

This osteolysis is thought to be immune responses. In this study, this foreign body reaction can be found in the biocompatibility test of CB1. A similar observation was reported in some study (7). In the latter study, an excessive foreign body reaction characteristic of encapsulation was evident. The xenografts still have some disadvantages in terms of graft incorporation, resorption, mechanical strength, and other problems linked to immunologic rejection and microbiologic contamination (15). Presently, groups CB1 and CB1bmp were encapsulated with a dense fibrous tissue with inflammatory cells such as neutrophils, macrophages, and foreign body giant cells at 4, 8, and 12 weeks after implantation. For this inflammatory reaction, freeze-drying after defatting was not suitable for CB.

In group CBHA, the most visible bone regeneration processing towards the centre of the defect was observed. The biocompatibility of CBHA echoes the findings of another study (3).

Gray-level density was measured on the basis of the standard radiographs, and the percentage of radiopacity was determined with a computer algorithm that divides the radiopaque area of the defect by the total area of the measuring frame (28). Presently, the radiologic gray-level histogram was measured for objective assessment of the radiologic findings using s similar principle. The gray-level histogram change in group CBHA during 12 weeks was the highest, indicating that bone regeneration in this group is most active, corroborating the results of radiologic analysis. However, there was

no significant difference in the gray-level histogram changes between groups CB1 and CB1bmp during 12 weeks. Evaluation of bone defect regeneration was measured objectively by gray-level histogram. The result supported that of a previous study (28).

To confirm the bone regeneration capacity, the defect closure of each group was measured 4, 8, and 12 weeks after implantation. The measurement of defect closure has been used before (14,20). The amount of newly-matured bone in the implanted material with rhBMP-2 was more than that evident without rhBMP-2 (17). In this study, after 8 weeks, group CB1bmp showed significantly higher result than group CBHA ($p < 0.05$). It is considered that the rhBMP-2 played a critical role in bone regeneration, but CB1 did not participate as the scaffold of rhBMP-2, considering the thickness of connective tissue. It is considered that rhBMP-2 contained in CB1 was freed to the surrounding bone tissue where it promoted the bone regeneration, but rhBMP-2 did not spread to the other implant sites. Regarding overall changes of defect closure, the bone regeneration capacity of CHA was the highest, followed by CBHA. Because this measurement of bone defect regeneration is invasive, the radiologic gray-level histogram will be a valuable method in clinical use. Advantages of CB and CBHA include their read availability, inexpensive cost, unlimited quantity and soft-textured surface. However, they suffer from weak strength. Conversely, CB and CBHA are easy to process and are suitable for use in irregular bone defects like sealing bone wax. Considering the biocompatibility of CBHA, the possibility that BMP acts as a scaffold is expected. Furthermore, CBHA will be able to be used by carrier or scaffold in stem cells. It was inconvenient not to have a study of CB2 + rhBMP-2.

Ideal bone substitutes should be resorbed with time and replaced by newly formed bone (23). In this study, newly formed bones and bone resorption were observed inside of graft material in group CBHA 12 weeks after implantation. But, there was no new bone formation in groups CB1, CB1bmp, and CB2. It is considered that the CBHA is a more biocompatible material in compact bone defect regeneration than CB1, CB1bmp, and CB2. However, further experiments are necessary to find out in more detail about the suitable absorptivity of CBHA.

To apply the most effective scaffold of bone graft substitute, various preparations of CB and different types (such as powder, paste, granule) of CBHA implantation should be studied.

Conclusion

This study was conducted to evaluate the biocompatibility of CB1, CB2, CBHA, and CHA using a mouse subcutaneous implant model and bone defect regeneration with CB1, CB2, CB1bmp, CBHA, and CHA in a rabbit calvarial defects model. The efficiency was compared with other commonly used CHA. The findings of this study indicate that CBHA is a safer material for use inside the body than CHA. CBHA has a more effective osteoconduction than CB1, CB1bmp, and CB2. CBHA is a valuable bone graft material in compact bone defect models.

Acknowledgement

This research was supported by the 2015 scientific promotion program funded by Jeju National University.

References

1. Ben-Nissan B. Natural bioceramics: from coral to bone and beyond. Curr Opin Solid State Mat Sci 2003; 7: 283-288.
2. Birchall JD, Thomas NL. On the architecture and function of cuttlefish bone. J Mater Sci 1983; 18: 2081-2086.
3. Chiroff RT, White EW, Weber JN, Roy DM. Tissue ingrowth of replamineform implants. J Biomed Mater Res 1975; 9: 29-45.
4. Choi IH, Lee CI. Effectiveness of transplantation by freeze-dried bone of goat to dogs. Korean J Vet Clin Med 1998; 15: 442-449.
5. Dupoirieux L, Pourquier D, Picot MC, Neves M. The effect of pentosan polysulphate on bone healing of rat cranial defects. J Craniomaxillofac Surg 1999; 27: 314-320.
6. Dupoirieux L, Pourquier D, Souyris F. Powdered eggshell: a pilot study on a new bone substitute for use in maxillofacial surgery. J Craniomaxillofac Surg 1995; 23: 187-194.
7. DurmuşE, Çelik I, Aydln MF, Ylldlrlm G, Sur E. Evaluation of the biocompatibility and osteoproductive activity of ostrich eggshell powder in experimentally induced calvarial defects in rabbits. J Biomed Mater Res Part B: Appl Biomater 2007; 86B: 82-89.
8. Flatley TJ, Lynch KL, Benson M. Tissue response to implants of calcium phosphate ceramic in the rabbit spine. Clin Orthop Relat Res 1983; 179: 246-252.
9. Gu SJ, Sohn JY, Lim HC, Um YJ, Jung UW, Kim CS, Lee YK, Choi SH, The effects of bone regeneration in rabbit calvarial defect with particulated and block type of hydroxyapatite. J Korean Acad Periodontol 2009; 39: 321-329.
10. Guillemin G, Patat JL, Fournie J, Chetail M. The use of coral as a bone graft substitute. J Biomed Mater Res 1987; 21: 557-567.
11. Huh JY, Choi BH, Kim BY, Lee SH, Zhu SJ, Jung JH. Critical size defect in the canine mandible. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2005; 100: 296-301.
12. Ivankovic H, Ferrer GG, Tkalcec E, Orlic S, Ivankovic M. Preparation of highly porous hydroxyapatite from cuttlefish bone. J Mater Sci: Mater Med 2009; 20: 1039-1046.
13. Itoh T, Mochizuki M, Nishimura R, Matsunaga S, Kadosawa T, Kokubo S, Yokota S, Sasaki N. Repair of ulnar segmental defect by recombinant human bone morphogenetic protein-2 in dogs. J Vet Med Sci 1998; 60: 451-458.
14. Jung SW, Jung JH, Chae GJ, Jung UW, Kim CS, Cho KS, Chai JK, Kim CK, Choi SH. The analysis of bone regenerative effect with carriers of bone morphogenetic protein in rat calvarial defects. J Korean Acad Periodontol 2007; 37: 733-742.
15. Karaismailoğlu TN, Tomak Y, Andaç A, Ergün E. Comparison of autograft, coralline graft, and xenograft in promoting posterior spinal fusion. Acta Orthop Traumatol Turc 2002; 36: 147-154.
16. Kim HS, Lee MY, Lee SC. Characteristics of sepiae os as a calcium source. J Korean Soc Food Sci Nutr 2000; 29: 743-746.
17. Kim JH, Kim CH, Kim KW. Bone healing capacity of the collagen bone filler (TERUPLUG®) and rhBMP-2 in rabbit

cranium defect. J Kor Oral Maxillofac Surg 2008a; 34: 119-130.
18. Kim, JJ, Kim HJ, Lee KS. Evaluation of biocompatibility of porous hydroxyapatite developed from edible cuttlefish bone. Key Engineering Materials 2008b; 361: 155-158.
19. Lee CH, Jang JH, Lee JM, Suh JY, Park JW. Histomorphometric evaluation of bone healing with natural calcium carbonate derived bone substitute in rat calvarial defect. J Korean Acad Periodontol 2008a; 38: 83-90.
20. Lee SK, Kim JS, Kang EJ, Eum TK, Kim CS, Cho KS, Chai JK, Kim CK, Choi SH. Effects of rhBMP-2 with various carriers on bone regeneration in rat calvarial defect. J Korean Acad Periodontol 2008b; 38: 125-134.
21. Lu JX, Flautre B, Anselme K, Hardouin P, Gallur A, Descamps M, Thierry B. Role of interconnections in porous bioceramics on bone recolonization in vitro and in vivo. J Mater Sci Mater Med 1999; 10: 111-120.
22. Okii N, Nishimura S, Kurisu K, Takeshima Y, Uozumi T. In vivo histological changes occurring in hydroxyapatite cranial reconstruction. Neurol Med Chir 2001; 41: 100-104.
23. Park JW, Bae SR, Suh JY, Lee DH, Kim SH, Kim H, Lee CS. Evaluation of bone healing with eggshell-derived bone graft substitutes in rat calvaria: A pilot study. J Biomed Mater Res 2008; 87A: 203-214.
24. Roy DM, Linnehan SK. Hydroxyapatite formed from Coral Skeletal Carbonate by Hydrothermal Exchange. Nature 1974; 247: 220-222.
25. Sohn JY, Park JC, Um YJ, Jung UW, Kim CS, Cho KS, Choi SH. Spontaneous healing capacity of rabbit cranial defects of various sizes. J Periodontal Implant Sci 2010; 40: 180-187.
26. Vuola J, Göransson H, Böhling T, Asko-Seljavaara S. Bone marrow induced osteogenesis in hydroxyapatite and calcium carbonate implants. Biomaterials 1996; 17: 1761-1766.
27. Weibrich G, Trettin R, Gnoth SH, Duschner HGH, Wagner W. Analysis of the size of the specific surface area of bone regeneration materials by gas adsorption. Mund Kiefer Gesichtschir 2000; 156: 1-5.
28. Zegzula HD, Buck DC, Brekke J, Wozney JM, Hollinger JO. Bone formation with use of rhBMP-2 (recombinant human bone morphogenetic protein-2). J Bone Joint Surg Am 1997; 79: 1778-1790.

토끼에서 오적골 이식 후 골 결손부 재생 평가

원상철 · 이주명 · 박현정 · 서종필 · 정종태[1]

제주대학교 수의과대학 · 제주대학교 수의과학연구소

요 약 : 골대체제는 지연유합이나 유합부전 그리고 골절술과 관절고정술 시 골편의 연속성 확립이 필요한 경우 골절의 주요 결손부위를 채우는데 주로 활용되고 있다. 본 연구에서는 오적골의 다양한 전처리 후 직경 5 mm 두께 2 mm의 형태로 가공하여 rhBMP-2의 담체로써 골전도력과 골유도력을 평가하고자 하였다. 결합조직의 두께는 2, 4주차 모두 오적골유래 hydroxyapatite 적용군(CBHA)에서 가장 유의성 있게 얇았다($p < 0.05$). Radiologic gray-level histogram의 측정에서는 4주차에서 CBHA군이 산호유래 hydroxyapatite 적용군(CHA)군보다 유의성 있게 높게 나타났으며($p < 0.05$), 12주차에서는 CHA군의 변화율이 가장 적었다. 전체 12주 동안의 변화율에서는 CBHA가 가장 많은 변화를 보였다. 폐쇄율에 있어서는 4, 8, 12주차 모두 CHA군이 다른 군에 비해 유의성 있게 높게 나타났으며($p < 0.05$), 8주차에서는 bmp를 적용한 오적골 적용군(CB1bmp)이 CBHA군보다 유의성 있게 높게 나타났다($p < 0.05$). 이상의 결과들은 CBHA가 생체 내에 적용하는 골대체재로서 골유도능력이 우수한 것으로 나타났다. 따라서 CBHA는 편평골에 있어 생체적합성이 뛰어난 골대체재로 그 가치가 있는 것으로 생각되며, 수의 임상에 있어서 활용성이 매우 높을 것으로 사료된다.

주요어 : 골 이식, 오적골, 수산화인회석, 산호, 발판