DOI: 10.4149/BLL_2012_119

Bratisl Lek Listy 2012; 113 (9)
529 – 533

EXPERIMENTAL STUDY

Radiological evaluation of the effect of biphasic calcium phosphate scaffold (HA+TCP) with 5, 10 and 20 percentage of porosity on healing of segmental bone defect in rabbit radius

Farahpour MR[1], Sharifi D[2], Gader AAB[3], Veshkini A[2], Soheil A[3]

Department of Clinical Science, Faculty of Veterinary Medicine, Islamic Azad University Uromia Branch, Uromia, Iran. **mrf78s@gmail.com**

Abstract: The objective of this study is to radiologically evaluate the effects of biphasic calcium phosphate scaffold with 5, 10 and 20 percentage of porosity on cortical bone repair in rabbits. In this study, 28 male white rabbits were examined. Rabbits were divided into four groups. After induction of general anesthesia, a segmental bone defect of 10 mm in length was created in the middle of the right radius shaft. In group A, the defect was stabilized with miniplate and 2 screws and left untreated. In groups B, C and D tricalcium phosphate scaffold mixed with hydroxyapatite (TCP+HA) with 5%, 10% and 20% porosity was used to fill the bone defect. Bone regeneration and HA+TCP scaffold resorption were assessed by X-ray at 1, 2 and 3 months after the surgery. In group A, 3 months after surgery, periosteal callus was not found but intercortical callus was observed. In groups B and C, 3 months after surgery medullary bridging callus and intercortical callus were found, periosteal callus was not found, TCP+HA scaffold were observed. In group D, 2 months after the surgery, medullary bridging callus and intercortical callus were found, 3 months later, periosteal callus was not found, most of scaffold had disappeared and were unclear and partial bone formation was recognized. Differences observed in radiological findings were significant between group A and groups B, C, D. Differences between groups B and C were not significant, but between group D and groups B and C were significant. The results of this study showed that TCP+HA scaffold is an osteoconductive and osteoinductive biomaterial. Scaffold of TCP+HA can increase the amount of newly formed bone and more rapid regeneration of bone defects. These results suggest TCP+HA scaffold may considerably be used in the treatment of cortical bone defect and other orthopaedic defects PCL *(Tab. 2, Fig. 4, Ref. 20)*. Full Text in PDF *www.elis.sk.*
Key words: radiology, HA+TCP, scaffold, bone healing, rabbit.

Fractures are one of the most important clinical problems in humans and animals. It has to be considered that skeletal system is related to peripheral nervous system on one hand, and soft tissues such as muscles around it on the other. It has an important physiological and anatomical role in movement system and considering the hypothesis of long recovery time or no recovery of fractures, many researches and studies have been done in the recent decades. In this regard, biomedical substances field of study has been developed to find the newest solutions to solve these problems.

The final goal of these studies is to gain weight ability, have ordinary movements, and natural physical activity of involved limb of the body that increases life time. But it should be noted that to develop the substance for transplantation, the reflection of the body must be taken into consideration.

Department of Clinical Science, Faculty of Veterinary Medicine, Islamic Azad University Uromia Branch, Uromia, Iran, [2]Departments of Clinical Science, Faculty of Veterinary Medicine, Tehran University, Tehran, Iran, and [3]Department of Radiology, Faculty of Specialized Veterinary Medicine, Islamic Azad University Science and Research Branch, Tehran, Iran

Address for correspondence: M.R. Farahpour, Department of Clinical Science, Faculty of Veterinary Medicine, Islamic Azad University Uromia Branch, Uromia, Iran
Phone: +98.441.4373676, Fax: +98.441.3460980

In this regard, the body would show reflections against transplantation, neutrality of substances, bioactivity reabsorbing of substance and the rejection of the substance (16). Nowadays bone transplantation and bone substitution in recovery of bone deficiencies on compressed or spongy bone are used.

The clinical result of transplantation method depends on many variants such as fracture locations, transplantation type, fixing type (7, 16). The substances used in bone transplantation can be divided into the following groups: autografts, alografts, zerografts and synthetic substances (2). With regard to the mentioned facts, excessive application of bone Grafts such as Alugens or Augens is restricted because of their rejection, infections and also high mortality. Nowadays synthetic substances are used to overcome the limitations instead of bone grafts. Calcium phosphates scaffold are new bioceramical substitutions for tissue engineering. Having the same mineral compounds and same size or distribution of porosity with bone make these substances the best substitutions for damaged bone in powder and solid form (9). Ingredient and porosity have been suggested as two important factors in optimum and effective functions of scaffold in bone engineering. Derivatives of calcium phosphate are famous synthetic materials that can be replaced by hard tissues (11). In these materials ratio of calcium to phosphorus is similar to natural materials in bone (4). There are several reports

about osteo guidance effects and absorption of calcium phosphate compounds in tissue defects (3, 6, 10, 14). Hydroxyapatite is a mineral matter and natural form of calcium apatite which exists in bone, tooth and sea coral naturally. Among properties and specialty of Hydroxyapatite as biomaterial, the most important specialty of Hydroxyapatite is a well bio adaptability (15). In reality Hydroxyapatit is a bio active material. Bioactivity of a material explains the ability of that material in connection with live tissue.

In addition to well bio adaptability, it seems that hydroxyapatite has direct chemical connection with hard tissues (1, 15, 20).

The goal of this research is the radiological study of osteo scaffold effects of 3-calciumphosphate that are mixed with Hydroxyapatite as nonorganic osteogenic substance with the porosity of 5, 10 and 20 percent which perhaps can be presented as one of the suitable compounds that can be used instead of bone grafts.

Materials and methods

Experimental study performed on 28, 6 months old, male News-Zealander rabbits, weight: 3 – 3.50 kg

Rabbits were kept in same conditions and divided into 4 groups of 7.

Group A as the control group and group B, C, D as the examining groups. In group A osteo defect was fixed by osteo plates, and in group B, C, and D osteo defect was filled with scaffold of 3-calciumphosphate mixed with Hydroxyapatite with 5, 70, 20 % porosity.

Surgery method

To create anesthesia ketamin hydroxy chloride (35 mg for per kg of body weight) (ketamine 10 %, Alfasan, woerden – Holland) mixed with xylazin (xylazin 2 % alfasan, worden Holland) (5 mg for per 1 kg of body weight) were used intravenously.

To simplify the anesthesia, Diazepam (1 mg for per 1 kg body weight) used intra muscularly as a pre-anesthesia; lateral anterior area of right anterior limb was prepared for a routine surgery.

In group A one parallel scission with 3 cm length was made with longitudinal axis of radious bone in lateral anterior surface of right anterior limb.

Fascia, tendon and connective tissue were kept away without cutting and damaging with engraving and anterior part of radious bone was seen.

Afterwards, middle part of radious bone was cut in 1 cm length with scalping iron.

Quickly after that, the location was washed with normal saline; two pieces of radious bone were fixed by 4-holed titanium osteo mini plate by using 2 screws (mizuhoco, Ltd. Tokyo, Japan). All over of fascia and under skin connective tissues were stitched with polyglycolate suture yarn 3-0 (polygiy collate, supobon, SUPA) and skin was stitched with a simple method using three zero nylon yarn (Nylong, CAT, GU, Iran).

In groups B, C and D like group A, one piece of radious bone with 1 cm length was separated, the space between the two parts of radious bone was filled with bony scaffold of 3-calciumphosphate mixed with Hydroxyapatite (technical, engineering medical Tehran Azad University) as the rate of scaffold porosity in group B was 5 percent, in group C 10 percent and in group D was 20 percent.

Fascia, under skin connective tissue and skin stitched like group A.

To avoid probable infection, 60000 unit G procaine penicillin was injected daily (Penicilin G procaine 400000, Zakaria, Tabriz, Iran) and 5 mg Gentamicin (Gentamicin, 20 mg/2 ml, zahravi, Tabriz, Iran) per kg body weight intramuscularly once a day for 5 days.

Radiologic evaluation

To evaluate the radiology of tissue responses in defected part, graphs were obtained in days 0, 30, 60, 90 after surgery using following factors:

KVP = 40 FFD = 100 cm MAS = 13

For this purpose, a radiograph was taken from lateral surface of radious bone with stable distances, quantity and quality of X-ray in all rabbits. In prepared radiographs, process of conglutination in bone defects was assessed (subjective Qualitative Evaluation).

Following indexes were evaluated for this purpose:

Amount of internal potential formed callus (medullary Bridging callus), amount of external potential formed callus (periosteal Bridging callus), amount of potential formed callus within the cortex (Intracortical callus), Stimulation of defected contiguous bones to start osteo production, the amount of opacity of scaffold and its role in osteoconductivity during the conglutination, evaluation of scaffold movement, evaluation the role of TCP + HA scaffold in defect place.

Some factors such as type of callus, radiological evaluation of conglutination, osteogenesis, grading and finally assessment of bone conglutination for each one areshown in Table1.

Statistical analysis

To analyze the data, the SPSS software (version 13) on win XP was used to compare the conglutination between groups in

Tab. 1. Ranking the role of TCP + HA scaffolding in place of Bone defect.

Grade	Classification
	A: Formed callus classes
0	No callus formation
1	External callus formation
2	Intercortical callus formation
3	Internal callus formation
4	Internal and intrcortical callus formation
	B: Radiological conglutination:
0	No sign of new bone formation
1	New bone formation sign of
2	adjacent bones stimulation and start of new bone formation
3	Imperfect conglutination
4	Perfect conglutination
	C: Osteogenesis
0	Lake of osteogenesis
1	Filling 25 % of bone defect
2	Filling 50 % of bone defect
3	Filling 75 % of bone defect
4	Filling 100 % of bone defect

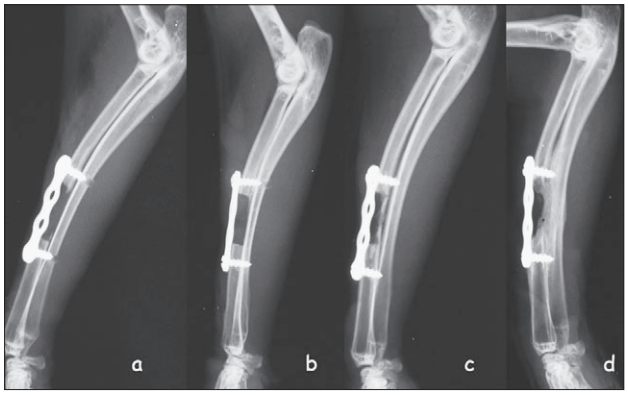

Fig. 1. Radiological assessment in group A. A) Radiograph after mini plate stabling, B) Radiograph 30 days after operation, no sign of callus formation, C) Radiograph 60 days after operation, a little intercortical callus formation, D) Radiograph 90 days after operation, noticeable intercortical callus formation.

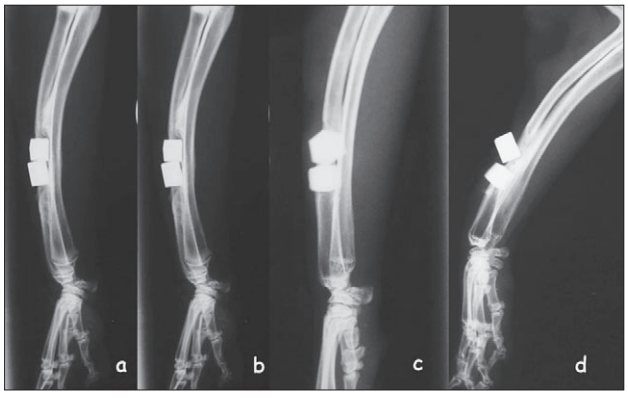

Fig. 2. Radiological assessment in group B. A) Radiograph after 5 % porosity HA&TCP scaffold implantation), B) Radiograph after 30 days of operation showed no movement or bone reaction), C) Radiograph after 60 days of operation showed little movement and internal callus was formed), D) Radiograph after 90 days of operation showed complete movement and both internal and intercortical callus were completely formed).

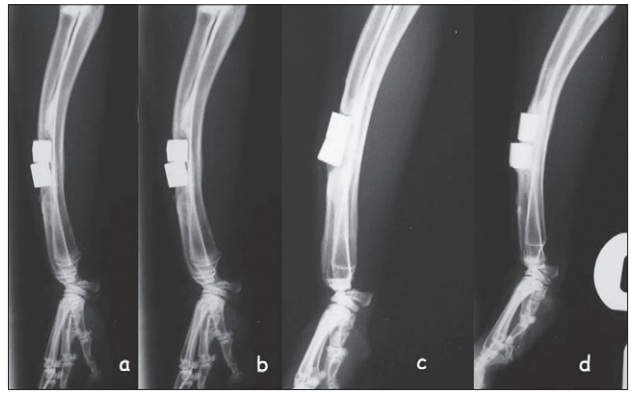

Fig. 3. Radiological assessment in group C. A) Radiograph after 10 % porosity HA&TCP scaffold implantation, B) Radiograph after 30 days of operation showed no movement and bone reaction was seen in radious bone margin), C) Radiograph after 60 days of operation showed no movement but internal and intercortical reactions were seen), D) Radiograph after 90 days of operation showed most internal and intercortical bone reaction and was completely attached to the ulnar.

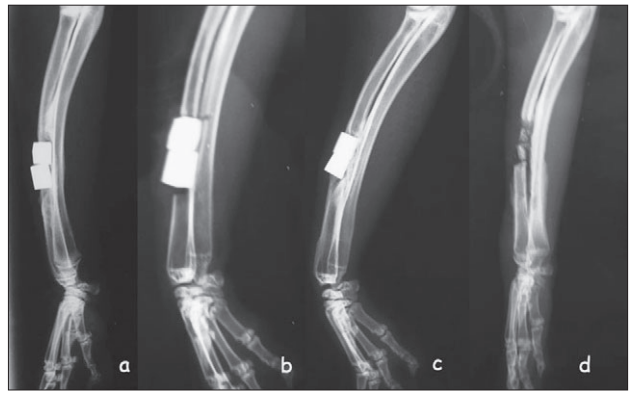

Fig. 4. Radiological assessment in group D. A) Radiograph after 20 % porosity HA&TCP scaffold implantation, B) Radiograph after 30 days of operation showed opacity change, internal and intercortical reaction were not seen), C) Radiograph after 60 days of operation showed no movement, internal and intercortical reactions were notably seen), D) Radiograph after 90 days of operation showed scaffold opacity decrease and it was completely mixed with callus tissue).

different periods of the examination, Kruskal–Wallis and Mann–Whitney U tests were used. To compare evaluation of groups two by two, Kruskal–Wallis test was used and variations were significant for $p < 0.05$.

Results

Evaluation of radiological results show that in group A after one month of stabilization of two pieces of radious bone by plates, osteo callus was not produced but after two months of surgery, very little intracortical callus had been produced. Taken Radiographs after three months of surgery show that no periosteal callus was produced in this group but noticeable amount of intercontical callus was shown (Fig. 1).

Comparison between group A grafts (the control group) and the examining groups shows that in group B after one month from transplanting HA & TCP with prosity of 5 %, no change occurred in scaffold opacity and no movement took place in scaffold transplant location and no osteo producing reaction was shown.

But after two months of surgery scaffold had a little movement from the transplanted location and medullary osteo calluse was being produced, movement of scaffold was obvious after 3 months and medullary & intracortical callus were produced and also no significant changes in opacity of cement have been shown and periosted callus was not shown (Fig. 2).

But in group C after one month of transplanting HA & TCP scaffold with the porosity of 10 %, osteo reaction on the edges of radius bone was started and the scaffold did not move from the transplant site.

After two months no movement took place in scaffold transplant location and the osteo reactions were shown in medullary & intracortical callus. After 3 months of surgery by progressing of osteo reaction between medullary and intracortical callus the

Tab. 2. Ranking quality parameters obtained.

Bone formation							Radiological assessment of bone healing							Formed callus classes								
7	6	5	4	3	2	1	7	6	5	4	3	2	1	7	6	5	4	3	2	1	Parameter Ratings	Month
																					Groups	
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	A	1
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	B	
0	0	0	0	0	0	0	1	1	1	1	1	0	1	0	0	0	0	0	0	0	C	
0	0	0	0	0	0	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0	D	
0	0	0	0	0	0	0	1	1	1	1	1	1	1	2	2	2	2	2	2	2	A	2
0	0	0	0	0	0	0	2	2	2	2	2	2	2	3	3	3	3	3	3	3	B	
0	0	0	0	0	0	0	2	2	2	2	2	2	2	4	4	3	4	3	3	3	C	
0	0	0	0	0	0	0	2	2	2	2	2	2	2	4	4	4	4	4	4	4	D	
1	1	1	2	1	1	1	3	3	2	3	2	3	3	2	3	2	2	2	3	2	A	3
1	1	1	1	1	1	1	3	3	3	3	3	3	3	4	4	4	3	4	4	3	B	
1	1	1	1	1	1	1	3	3	3	3	3	3	3	4	4	4	4	4	3	4	C	
2	3	3	3	3	2	2	3	3	3	3	3	4	3	4	4	4	4	4	4	4	D	

scaffold was surrounded, and the attachment of scaffold to edges of the radial bones was completed.

Severe reduction in opacity of scaffold, color fading, and making more porosity in scaffold show complete mixture of host tissue and the scaffold. There was no movement in transplant location and the produced callus had brick-like shape (Fig. 3) bone as well as opacity was changed.

In group D, HA-TCP scaffold transplanting with 20 % porosity, movement at transplant location did not occure after one month and in the first month opacity reduction was not seen, but there were no intracortical and medullary osteo reactions. There was no movement and more opacity reduction was seen after two months and intracortical and medullary reactions were obvious. After three months the scaffold opacity was reduced a lot and there was more porosity and the scaffold was completely mixed with callus tissue (Fig. 4).

Qualitative factors achieved from this examination include:

Type of the produced callus, evaluation of conglutinative radiology and amount of osteogenesity are ranked in Table 2. The results were analyzed statistically. Statistical evaluation of the results shows positive effect of TCP + HA scaffold in group B, C, D compared to group A on days 0, 90 after surgery and changes are significant ($p < 0.05$).

Results from Group B and C show no significant changes between these groups and comparison between the results of group D with group B and C shows significant changes on days 60 and 90 after surgery.

Conclusion

In this comparative study of prepared radiographs from the controlled and examined groups, the reason of callus absence can be considered a lack of bone ends stimulation or stability of fracture. Due to the fact that bone ends movement during the conglutination process causes osteogenic effects on external callus production, the absence of external callus o radiographs shown above can be due to the fracture stability. This study has not the power to r define regeneration time as well as long distance between two ends of fracture and lack of natural pattern of fracture. Presence of intracortical and internal callus in examined groups after three months of operation can be explained because of bone stimulation by calcium phosphate and hydroxyappatite scaffold when compared with the control group, also the production of callus in examined groups was higher than control group. Firm fixation of bones will definitely reduce osteogenic activity in bone periosteum, in fact it will prevent peristeal reaction and the external callus will not regenerate, but any movement, even insignificant, between two ends of bone will stimulate callus formation, particularly the external callus. Present results show that the bone scaffold is effective and has stimulated fractured bone and bone regeneration which is showed by the osteoinduction effect of 3-calciumphosphate and hydroxyapatite. Porosity is the main factor in osteoconduction effect of scaffold which causes formation and expansion of osteogenic cells through this porosity, and if the porosity percentage would increase it could cause higher conductibility and inductivity of scaffold. Considering this, in group B with 5 % porosity new born bone couldnot infiltrate to the scaffold and has moved it.

In the study by Ignjatovic et al (2007) which was done by biphasic calcium phosphate cover using biomaterials such as Poly-D-, L- Lactide-co-Glycolide as a bone substitution, they showed that calcium phosphate and hydroxyapatite had a main effect on fibroblasts accumulation and adhesion of them to these substances which prepare condition in fracture location(8).

Daculsi et al (2006) used biphasic calcium phosphate ceramic accompanied with cow bone zenograft in rat bone regeneration process, and showed that due to increased stimulation and calcium assembling at the location the best quality because of hydroxyapatite was obtained (5). In the other study by Motomia et al (2007) which was done on lumbar vertebra of 36 rabbits, hydroxyapatite with 15, 50, 85 % porosity accompanied with autogenous bone was used and showed hte osteogenesis rate in 85 % hydroxyapatite group was significantly higher than in the other two groups(13). In a clinical study by Weissman et al (1996) hydroxyapatite was used in order to correct bone defects in 24 patients jaws and prepared radiographs showed that hydroxyapatite cement has completely

affected stimulation of bone regeneration (19). In a radiological study by Menon and Varma (2005) using hydroxyapatite on fractured tibia of 28 patients and preparing continuous radiographs from fractured location, it was concluded that hydroxyapatite is a biomaterial which can be easily used and can cause bone stimulation in fractured location as well (12).

Sharify et al (2007) carried out another study of calcium phosphate accompanied with collagen type-I, and showed that in a 3-month period some amounts of calcium phosphate have been absorbed and the external and intracortical callus was produced. Regarding the fact that calcium phosphate cement is a biocompatible substance with body and has osteoconductivity characteristic, collagen type-I augmentation has important roles in osteoinduction, osteogenesis and osteogenic cells increase by internal and intracortical callus reactions though(17).

In a study by Komaki et al (2006) which have evaluated beta calcium phosphate and fibroblast growth factor-2, it was shown that beta calcium phosphate cement increases osteogenesis during 3months in rabbit tibia bone and adding fibroblast growth factor-2 at the same time, caused osteoinduction and osteogenesis in the examined group (9). Considering the stimulative and accelerative characteristics of 3-calciumphosphate it seems that whenever a substance with osteoinduction characteristic is added to 3-calciumphosphate it can be used in bone defections. Turner TM et al (2003) reported that by using a type of scaffold which is called proosteon 200R with some porosity, bone growth rate will be significantly higher than local reaction of cement alone, and during 6 to 8 weeks main part of cement will be absorbed by host tissue infiltration (18). Results show that scaffold has better osteoinduction and osteoconduction has a lot of porosity compare to osteoplate.

It seems that the principle of graft and bone substitution by cement substances or biological scaffolds is all about ability of these substances in fixing the fractured bone ends, graft and bone defects. It is difficult to fix fractured bone in front of each other and eliminate any spaces between them in normal conditions both in human and animals and most often movement in the location stimulates the external callus and occasionally cause ruptures in new vessels of newborn tissue following that new thrombosis will be formed which complicates callus formation process with different maturity and age. Anyhow, by fracture fixation simultaneously fractured location can be filled by these materials which can help to fix fractured bone ends as well as to make a foundation to osteogenic cells where these cells can easily grow throughout them and as time passes graft fractured bone ends or margins of bone defects. Studying present results shows that 3-calciumphosophate and hydroxyapatite scaffolds are body adapted biomaterials, and 3-calciumphosophate scaffold with osteoinduction and osteoconduction abilities can fix two ends of bone to each other and increases conglutination process, it can be combined with 3-calciumphosophate as well .This compound can increase ostoeoinduction, osteoconduction and osteogenesis function. Considering the mentioned matters, this method can be used in open, close and new fractures even in fractures or defections which have been treated by cortical bone but did not regenerate properly.

References

1. Bauer TW, Muschler GF. Bone graft materials. An overview of the basic science. Clin Orthop 2000; 371: 10 – 27.

2. Block JE, Poser J. Does Xenogeneic demineralized bone matrixes have clinical utility as a bone graft substitute? Med Hypotheses 1995; 45: 27 – 32.

3. Chazono M, Tanaka T, Komaki H, Fujii K. Bone formation and bioresorption after implantation of injectable β-tricalcium phosphate granules-hyaluronate complex in rabbit bone defects. J Biomed Mater Res 200; 70: 542 – 549.

4. Constansz BR, Flumer MT. Skeletal repair by insitu formation of mineral phase of bone. Science 1995; 267: 1796 – 1798.

5. Daculsi G, Corre P, Malard O, Legeros R, Goyenvalle E. Performance for bone ingrowth of Biphasic calcium phosphate ceramic versus Bovine bone substitute. Key Engineering Materials 2006; 309-311(Part 1 – 2): 1379 – 1382.

6. Dong J, Uemura T, Shirasaki Y, Tateishi T. Promotion of bone formation using highly pure porous β-TCP combined with bone marrow-derived osteogenitor cells. Biomaterials 2002; 23: 4493 – 4502.

7. Giannoudis PV, Dinopoulos H, Tsiridis E. Bone substitutes: an update. Injury 2005; 36: S20 – 27.

8. Ignjatovic N, Nikov P, Ajdukovic Z, Vasiljevic-Radovic D, Uskokovic D. Biphasic calcium phosphate coated with poly-D,L-lactide-co-glycolide biomaterial as a bone substitute. J Eur Ceramic Soc 2007; 27: 1589 – 1594.

9. Komaki H, Tanaka T, Chazono M, Kikuchi T. Repair of segmental bone defects in rabbit tibiae using a complex of β-tricalcium phosphate, type I collagen, and fibroblast growth factor-2. Biomaterials 2006; 27: 5118 – 5126.

10. Kondo N, Ogose A, Tokunaga K, Ito T, Arai K, Kudo N. Bone formation and resorption of highly purified beta-tricalcium phosphate in the rat femoral condyle. Biomaterials 2005; 26: 5600 – 5608.

11. Kurashina K, Kurita H, Hirano M, Kotani A. In vivo study of calcium phosphate cement: Implantation of a α-tricalcium phosphate/ dicalcium phosphate dibasic/ tetracalcium phosphate monoxide cement paste. Biomaterials 1997; 18: 539 – 543.

12. Menon KV, Varma HK. Radiological outcome of tibial plateau fractures treated with percutaneously introduced synthetic porous Hydroxyapatite granules. Eur J Orthop Surg Traumatol 2005; 15: 205 – 213.

13. Motomiya M, Ito M, Takahata M, Kadoya K, Irie K, Abumi K, Minami A. Effect of Hydroxyapatite porous characteristics on healing outcomes in rabbit posterolateral spinal fusion model. Eur Spine J 2007; 16: 2215 – 2224.

14. Ogose A, Hotta T, Hatano H, Kawashima H, Tokunaga K, Endo N. Histological examination of beta-tricalcium phosphate graft in human femur. J Biomed Mater Res 2002; 63: 601 – 604.

15. Ozturk A, Yetkin H, Memis L, Cila E, Bolukbasi S, Gemalmaz C. Demineralized bone matrix and hydroxyapatite/tri-calcium phosphate mixture for bone healing in rats. Intern Orthopaed 2006; 30: 147 – 152.

16. Petite H, Viateau V, Bensaid W, Meunier A, de Pollak C, Bourguignon M. Tissue engineered bone regeneration. Nat Biotechnol 2000; 18: 959 – 963.

17. Sharifi D, Mousavi G, Hasaraki S, Rabiee SM. Evaluation of Compressive Properties of the Radial Bone Defect Treated with Mixture of Calcium Phosphate and collagen type I in Rabbit. Iran J Veterinary Surg (in press).

18. Turner TM, Urban RM, Gitelis S, Haggard WO, Richelsoph K. Resorption evalution of a large bolus of calcium sulfate in a canine medullary defect. Orthopedics 2003; 26: 577 – 579.

19. Weissman JL, Snyderman CH, Hirsch BE. Hydroxyapatite cement to repair skull base defects: radiologic appearance. Amer J Neuroradiol 1996; 17: 1569 – 1574.

20. Zambonin G, Grano M. Biomaterials in orthopaedic surgery: Effects of different hydroxyapatites and demineralized bone matrix on proliferation rate and bone matrix synthesis by human osteoblasts. Biomaterials 1995; 16: 397 – 402.

Received April 16, 2011.
Accepted June 26, 2012.