Original Article

Addition of fresh autogenous bone marrow aspirate to bioglass accelerates bone formation in critical-size rabbit calvarial defects

Mohammad Mehdi Jahani[1], Mohammad Mehdi Dehghan[2*], Sarang Soroori[2], Saeed Farzad-Mohajeri[2], Azita Azimi Meibody[3]

[1]Faculty of Veterinary Sciences, Shahrekord Branch of Islamic Azad University, Shahrekord, Iran, [2]Department of Surgery & Radiology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran, [3]Faculty of Medicine, Najafabad Branch of Islamic Azad University, Najafabad, Iran.

Correspondence: Mohammad Mehdi Dehghan, Department of Surgery & Radiology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran. E-mail: mdehghan@ut.ac.ir

ABSTRACT

Introduction: We investigated the effects of adding bone marrow aspirate to bioglass in repair of rabbit calvarial bone defects. Methods: In this experiment 4 defects were created in each calvarium of the 9 rabbits, and each calvarium defect separately treated by autologous bone marrow mixed with bioglass, bioglass, autologous cancellous bone, and the fourth defect remained without treatment as control. Radiological, histological and histomorphological healing process assessed 4, 8 and 12 weeks postoperatively. Results: Cancellous bone treatment showed the best results radiographically and histologically. Control group showed the lower numbers. Bioglass and bioglass-bone marrow treatments were not enough effective as the cancellous bone treatment during the experiment time line. Conclusion: Although combination of bioglass and autologous bone marrow has a better osteoinductive and osteoconductive properties than bioglass by itself, but in comparison with autogenous cancellous bone, it still has a weaker and poorer osteoinductive and osteoconductive properties.

Keywords: Bone regeneration, bone marrow, Bioglass, cancellous bone, Rabbits.

Introduction

Bones have complex tissue and highly ordered structures. In micro-view they have fewer cells and most of the matrix fills by intercellular collagen fibers and strength materials [1]. In fractures, bone injuries and many situations, athwart other tissues, bones can restore themselves without scar making. But, in pathological fractures or large and heavy bone defections, recovery and repair of bones were unsuccessful. Infection of the bone or the surrounding tissues, lack of blood supply, and systemic diseases defect bone healing, and it caused delayed bone reunion or ineffectual of bone union [2].

Bone defects, caused by trauma or surgically for treatment of diseases, are a challenging concern for surgeons. Current treatment strategies include implantation of synthetic materials and transplantation of natural grafts. Bone grafting is a surgical treatment that replaces lost or damaged bones [3]. application of bone grafts have effective and accelerating impacts in treatment of bone damages [4]. However, limitation of current treatment plans have leads researchers to focusing their works for finding reliable bone graft donor sites and materials in clinical and surgical interventions [5-7].

Bone grafts have a key role to make a supportive structure in treatment of bone damages [8]. Bone grafts act as a scaffold to bone reconstruction and filler for bone defects of surgical operation or traumas, which named osteocunduction [4]. They help osteoblasts to improve their reproduction and separation in damaged parts resultred in osteogenesis and osteoinduction, respectively [9]. Despite the fact that, basic studies has resulted

Access this article online	
Website: www.japer.in	**E-ISSN:** 2249-3379

How to cite this article: Mohammad Mehdi Jahani, Mohammad Mehdi Dehghan, Sarang Soroori, Saeed Farzad-Mohajeri, Azita Azimi Meibody. Addition of fresh autogenous bone marrow aspirate to bioglass accelerates bone formation in critical-size rabbit calvarial defects. J Adv Pharm Edu Res 2019;9(S2):27-32.
Source of Support: Nil, Conflict of Interest: None declared.

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-Non Commercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

in the incorporation of several allografts, xenografts, and alloplastic materials into the routine methods, until now, in most cases autologous bone grafting is considered as the gold standard option [5]. In addition, Autologous grafts have no adverse immune reactions; so autologous graft in most cases is the most effective graft to bone treatment [10]. However, application of autologous graft has some disadvantages e.g donor site injuries and limited amount of extractable graft [3, 4].

To repairing the bone damages, Current researches focuse on different natural living grafts and synthetic non-living materials such as bone marrow [11], autologous bone slices and parts [12], stem cells [13], calcium based materials [14], silica based materials [15] etc. The purpose of this study is to describe radiographic and histomorphometric results of bioglass, bone marrow and bioglass-bone marrow in treatment of calvarial bone defects in rabbit.

Methods:

Animal model, groups and surgery

Nine male New Zealand white rabbits (NZWR) whit average weight of 2.5 kg were kept in separate cages. There were four different treatments in this experiment. The following groups were compared: 1) autologous bone marrow mixed with bio-glass 2) bioglass 3) autologous cancellous bone and 4) control empty group (no treatment). The local ethics committee and animal welfare approved the design of the experiment. No death or complication observed in animals during the study.

The rabbits were anesthetized by intramuscular injection of 35mg/kg ketamine (Ketamine 10%, Alfasan, Woerden, Holland) and 5 mg/kg xylazine (Xylazine 2%, 10%, Alfasan, Woerden, Holland). The anesthesia was continued with 1.5% isoflurane (Forane, Aesica Queenborough, Queenborough, Kent, UK) after endotracheal intubation and presurgical preparation was done for aseptic surgery. A midfrontal skin incision was made and the calvarium was exposed using a periosteal elevator. Four circular defects in same shape, thickness and a size made in each calvarium of the rabbits using a high speed burr (NSK, Nakanishi inc, Kanuma, Japan) with 5 mm diameter trephine under irrigation with 0.9% sterile saline solution. All defects standardized with anatomical landmarks that include occipital appendage, craniocaudal suture and sagittal suture (Fig. 1).

Then, Jamshidi biopsy needle used to extract 2 ml bone marrow from the greater tubercle of humerus of the same rabbit. Furthermore, a hole was created in the same region and cancellous bone extracted using a bone curette. Calvarial defects separately filled with autologous bone marrow mixed with bio-glass (Bioglass®, US Biomaterials, Alachua, FL, USA), bioglass, autologous cancellous bone, and the fourth defect remained without treatment as control.

Radiological, histological and histomorphological healing process evaluated 4, 8 and 12 weeks postoperatively. For this, each times, three rabbits were euthanized and harvested calvarium prepared for radiography immediately.

Radiological investigation:

Post mortem, using an autopsy bone saw each calvarium separated by four cut; each contains the frontal and parietal bones and includes created defect. radiographical image were taken in the dorso-ventral views of the bones. Radiographs were compared subjectively and quantified based on graded scaling. So, number 1 gives to the third or lesser; number 2 gives to third to the two-thirds and number 2 gives to two-thirds to full defect (hole) filling. Moreover, to quantifying the radiological results of the treated area unity, number 1 gives to completely osteogenic differentiation, number 2 gives to muffled osteogenic differentiation and number 3 gives to loss ofosteogenic differentiation. Also, densitometric evaluation of radiographs was examined by use of a stepped aluminum phantom with specified thickness as an index in each radiographs and image processing software (ImageJ, ImageJ Developers, National Institute of Health, USA). Furthermore, number 1 gives to the almost radiolucent defects, number 2 gives to till one-half the normal bone density and number 3 gives to almost equal with the normal bone density in defected area, to quantifying the radiological results of the treated area bone opacity.

Histological and histomorphological healing process evaluation

After radiography; bones fixed in 10% formalin (Merck Inc, Germany) for 10 days. The samples are transferred to solution containing 20% formic acid (Merck Inc, Germany) for 72 hours to decalcification process. Then for neutralization bones treated by 20% lithium carbonate (Merck Inc, Germany) solution in 5 minutes, and using microtome device bone sections prepared, coded (with India ink) and set for dehydration and clearing process. Afterward of serial dehydration, the marked bone sections placed into the paraffin blocks. From paraffin blocks belong to each defect, 25 - 30 cutting (perpendicular to cranial vault) with 5 µm diameter prepared, also stained with Hematoxylin and Eosin (H & E) method, and after that samples was evaluated by light microscope.

Bone formation percentage and the remaining biomaterial percentage are quantitative variables that were measured. And all prepared sections of any defect were photographed by a digital camera at magnification X40. For those assessments, image processing of resulting images (in JPEG format) implemented by Sigma Scan Pro 5 (Systat Software Inc.) software.

Statistical analyses

Statistical analysis was performed using SPSS 16 software (IBM Corp, New York, USA) and Tukey test was used for comparisons using a confidence level of $P < 0.05$ to determine the significance of differences.

Result:

Radiological results:

Defects treated by cancellous bone treatment showed the best results in all the times. Also control showed the lower numbers and bioglass and bioglass-bone marrow treatments were not enough effective as the cancellous bone treatment during the experiment time line (Figure 2 and Table I).

As the results shown in Table I, defect unity of the bone that treated by cancellous bone and also control showed the lowest numbers in all the times, in fact they show not significant unity to the surrounding bones. In the other hand, bioglass and bioglass-bone marrow treatments showed the promising results. However, bioglass-bone marrow treatment showed the best scores during the weeks of the experiment.

As Bone opacity mostly caused by mineral content and concentrations, so Increased in bone opacity is associated with increased mineralization or new bone productions [16]. As a result and based on the declared data in Table I, defect opacity treated by cancellous bone had the best results in all the intervals. Also control showed the lower numbers and bioglass and bioglass and bone marrow treatments not enough effective as well as the cancellous bone treatment during the experiment. But that cannot be seen, bioglass-bone marrow treatment always showed promising results and a little lower than results of cancellous bone treatment.

Histomorphometrical analysis and histomorphological analysis

As the results shown in Table II, cancellous bone treatment showed the best bone regeneration percentage in all the times; after 4 weeks of the treatments, cancellous bone are 5 time more effective than the control in bone regeneration and healing of the rabbits. Also control showed the lower numbers and bioglass and bioglass-bone marrow treatments not enough effective as well as the cancellous bone treatment during the experiment. Moreover, those efficiency ranks of the treatments in bone regeneration were unalterable after 8 and 12 weeks of the test.

After 4 weeks, in control defect (hole) samples, limited bone growth and immature woven bone trabeculae observed. In the center place of healing (defect center) immature woven bone trabeculae was quite thin and narrow, also scattered multiple limited foci of osteoid formation observed (Fig. 3, part A). In the control defects after 8 weeks, in defect area and also between bones trabeculae, the relatively mature connective tissue with small blood vessels, abundant connective cells and less inflammatory cells were completely visible. In that stage of healing intensity of the inflammatory response reduced and, the size and thickness of trabeculae was expanded; moreover of that the mineralization had begun (Fig. 3, part B). After 12 weeks in conrol group bone formation was better than 4 and 8 weeks. (Fig. 3, part C).

After 4 weeks, in bioglass treated defect samples, apparently 25% of the bioglass absorbed and surrounded by mononuclear phagocytic cells (monocytes or macrophages), polymorphonuclear cells and connective tissue. Bone growth and early woven bone trabeculae observed. That woven bone trabeculae was quite thin and narrow; but many bone trabeculae particularly mineralized and also, so similar to the osteoid (Fig. 4, part A). In the bioglass treated defects after 8 weeks, half of the bioglass absorbed. In the defect area and also between bones trabeculae, the relatively mature connective tissue with limited blood vessels, abundant connective cells and less inflammatory cells were completely visible. In that stage of healing intensity of the inflammatory response reduced and, the size and thickness of trabeculae was expanded; moreover, the created trabecula surrounded by giant and activate osteoblasts (Fig. 4, part B). After 12 weeks, bioglass reduced, , mature spicules changing to the lamellar bone formation with visible haversian systems or bone marrow cavities, and also bone induction was better than weeks 4 and 8 (Fig. 4, part C).

After 4 weeks, in autogenic cancellous bone treated defect group, new trabecular bone formation In the cener of defect was determined. (Fig. 5, part A). After 8 weeks of treatment, New bone trabeculae was thicker and larger than in 4 weeks; (Fig. 5, part B). After 12 weeks, there was ditinct complete new bone formation in the defect (Fig. 5, part C).

After 4 weeks, in bioglass-bone marrow treated defect samples, histo-pathological overview seems between bioglass treated defects and autogenic cancellous bone treated defects; but mostly look like bioglass treated defects. Bone growth and early woven bone trabeculae also observed. (Fig. 6, part A). The woven bone trabeculae were quite thin and narrow, moreover of that, some of the bone trabeculae particularly mineralized. After 8 weeks, half of the bioglass absorbed and observable changes similar to the same time stage of bioglass treated defects (Fig. 6, part B); Also, respectively after 12 weeks of the experiment (Fig. 6, part C).

Discussion:

The hypothesis for this study was that bone marrow aspirate promotes bone healing in a critical size calvarial defect on Bioglass scaffold. The data show that the addition of bone marrow had a significantly positive effect on bone healing, both radiographically and histologically. However, the effect was not as similar as cancelous bone graft.

Early studies have shown that autologous graft, especially autogenous bone-grafting are more effective in most situations [17-19]. Generally, the successful autogenic bone grafting depends on the rapid reproduction and survival of the bone cells, conditions at the receiving tissue, type of chosen graft or grafts, graft treatment, and the graft forming during the surgery method. Fresh autologous graft capable to support new bone growth in four ways: induction, genesis, conduction, and integration. Although some grafts may not have all of these characteristic, but they can be clinically effective [2, 20].

Previous studies reported the ability of bioglass to promote bone healing [21, 22]. In addition, some reports have shown that after implanting of bone scaffolds and substitutes, the bone density and healing are significantly greater when they are used in combination with bone marrow aspirate [23-25]. Bone marrow aspirate contains different type of cells especially mesenchymal stem cells [26].

Recent studies revealed Mesenchymal stem cells especially those derived from bone marrow, promote bone healing [27-30]. Common sources of obtaining mesenchymal stem cells are: Cultivation of these cells, peripheral blood and autogenous bone graft. As cultivation of Bone marrow needs special facilities, take prolonged time and has extra expenses for patients, bone marrow aspirates could be a suitable and available source of these cells [31] Furthermore, Bone marrow aspirate contains a higher percentage of mesenchymal stem cells than peripheral blood [26]. In addition, it is less invasive than harvesting of autogenous bone graft, is relatively easy with less pain and does not involve significant risks of complications [32].

In our study defected calvarium of 9 rabbits treated by autologous bone marrow mixed with bioglass, autologous cancellous bone, and bioglass. Radiological, histological and histomorphological investigations of healing process that evaluated after 4, 8 and 12 weeks of treatments showed that, the used osteo-grafts were effective. Weitao et al. [33] studied two different osteo-grafts, autologous iliac crest bone particles and injectable calcium phosphate cement. They also mentioned that the autogenous graft has better effects on bone healing in rabbits. Moreover, the grafts include autogenous wounded body parts were more effective than others. Kang et al. [19] compare the bone regeneration potential of two grafts, cultured allogeneic bone marrow and of autologous bone marrow loaded onto allogeneic cancellous bone. And that was in agreement with Kang et al. [19] findings. Also the graft was has more histological similarities (autologous cancellous bone) showed the best results of healing. In earlier study, Saad et al. [17] used the rabbit model for radiographic and histological evaluation of bone reconstruction using a calcium phosphate graft and when loaded with autogenous bone marrow-derived. So their results showed that fact. The results cleared that, defects treated by bioglass-bone marrow showed better healing signs than bone marrow treated sites; but almost all their of healing results were lower than defects treated by autologous cancellous bone. These results could be caused by these facts; firstly, bioglass-bone marrow treatment has more histological similarities than bone marrow treatment [17], in the other hand, it has less autogenous parts than autologous cancellous bone treatment [33].

Acknowledgements:

This project was performed by grants provided by University of Tehran and Institute of Biomedical research. The authors would like to thank Dr Hossein Gholami for helping in revision of histopathological results.

References

1. Brookes M, Revell WJ. Blood Supply of Bone: Scientific Aspects. Springer London; 1998. 359 p.
2. Oryan A, Alidadi S, Moshiri A, Maffulli N. Bone regenerative medicine: classic options, novel strategies, and future directions. J Orthop Surg Res. BioMed Central; 2014 Mar 17;9(1):18.
3. Uchida R, Toritsuka Y, Mae T, Kusano M, Ohzono K. Healing of tibial bone tunnels after bone grafting for staged revision anterior cruciate ligament surgery: A prospective computed tomography analysis. Knee. Elsevier; 2016 Oct 1;23(5):830–6.
4. Rinaldi M, Ganz SD, Mottola A, Lauriola S. Computer-guided applications for dental implants, bone grafting, and reconstructive surgery. Elsevier; 2016.
5. Bernades Mayordomo R, Guijarro Martínez R, Hernández Alfaro F. The anterior maxilla as a potential source of bone grafts: a morphometric cone beam computed tomography analysis of different anatomical areas. Int J Oral Maxillofac Surg. Churchill Livingstone; 2016 Aug 1;45(8):1049–56.
6. Troeltzsch M, Troeltzsch M, Kauffmann P, Gruber R, Brockmeyer P, NormanMoser, et al. Clinical efficacy of grafting materials in alveolar ridge augmentation: A systematic review. J Cranio-Maxillofacial Surg. Churchill Livingstone; 2016 Oct 1;44(10):1618–29.
7. Dao AM, Goudy SL. Cleft Palate Repair, Gingivoperiosteoplasty, and Alveolar Bone Grafting. Facial Plast Surg Clin North Am. Elsevier; 2016 Nov 1;24(4):467–76.
8. Johnson EO, Troupis T, Soucacos PN. Tissue-engineered vascularized bone grafts: Basic science and clinical relevance to trauma and reconstructive microsurgery. Microsurgery. 2011 Mar;31(3):176–82.
9. Bose S, Roy M, Bandyopadhyay A. Recent advances in bone tissue engineering scaffolds. Trends Biotechnol. 2012 Oct;30(10):546–54.
10. Sen MK, Miclau T. Autologous iliac crest bone graft: Should it still be the gold standard for treating nonunions? Injury. 2007 Mar;38(1): S75–80.
11. Wang T, Wang W, Yin ZS. Treatment of osteonecrosis of the femoral head with thorough debridement, bone grafting and bone-marrow mononuclear cells implantation. Eur J Orthop Surg Traumatol. 2014 Feb 6;24(2):197–202.
12. Ieong E, Mahapatra P, Nathan S. Fashioning autologous bone graft from the fibula in the transfibular approach to open ankle arthrodesis. Foot Ankle Surg. 2014 Jun;20(2):149–50.
13. Manassero M, Viateau V, Deschepper M, Oudina K, Logeart-Avramoglou D, Petite H, et al. Bone regeneration in sheep using acropora coral, a natural resorbable scaffold, and autologous mesenchymal stem cells. Tissue Eng Part A. 2013 Jul;19(13–14):1554–63.

14. Bernhardt A, Lode A, Peters F, Gelinsky M. Comparative evaluation of different calcium phosphate-based bone graft granules - an in vitro study with osteoblast-like cells. Clin Oral Implants Res. 2013 Apr;24(4):441–9.
15. McBride D, Clark JP, Davis R, Pomrink GJ, TOSUN Z, Greenspan DC. System and kit for delivering collagen bioglass composite bone grafting materials for regenerating hard tissues. US 9199032 B2, 2014.
16. Kealy JK, McAllister H, Graham JP. Diagnostic radiology and ultrasonography of the dog and cat. Saunders; 2011. 580 p.
17. Saad KA-E, Abu-Shahba AGT, El-Drieny EA-E, Khedr MS. Evaluation of the role of autogenous bone-marrow–derived mesenchymal stem cell transplantation for the repair of mandibular bone defects in rabbits. J Cranio-Maxillofacial Surg. 2015 Sep;43(7):1151–60.
18. Dasmah A, Kashani H, Thor A, Rasmusson L. Integration of fluoridated implants in onlay autogenous bone grafts – An experimental study in the rabbit tibia. J Cranio-Maxillofacial Surg. 2014 Sep;42(6):796–800.
19. Kang S-H, Chung Y-G, Oh I-H, Kim Y-S, Min K-O, Chung J-Y. Bone regeneration potential of allogeneic or autogeneic mesenchymal stem cells loaded onto cancellous bone granules in a rabbit radial defect model. Cell Tissue Res. 2014 Jan 30;355(1):81–8.
20. Vittayakittipong P, Nurit W, Kirirat P. Proximal tibial bone graft: the volume of cancellous bone, and strength of decancellated tibias by the medial approach. Int J Oral Maxillofac Surg. 2012 Apr;41(4):531–6.
21. Dorea HC, McLaughlin RM, Cantwell HD, Read R, Armbrust L, Pool R, et al. Evaluation of healing in feline femoral defects filled with cancellous autograft, cancellous allograft or Bioglass. Vet Comp Orthop Traumatol. 2005;18(3):157–68.
22. Teófilo JM, Brentegani LG, Lamano-Carvalho TL. Bone healing in osteoporotic female rats following intra-alveolar grafting of bioactive glass. Arch Oral Biol. 2004 Sep;49(9):755–62.
23. Chauhan V, Sharma S, Maheshwari R, Juyal A, Raghuvanshi S, Bansal S. Evaluation of hydroxyapatite and beta-tricalcium phosphate mixed with bone marrow aspirate as a bone graft substitute for posterolateral spinal fusion. Indian J Orthop. 2009 Jul;43(3):234.
24. Soltan M, Smiler D, Prasad HS, Rohrer MD. Bone Block Allograft Impregnated with Bone Marrow Aspirate. Implant Dent. 2007 Dec;16(4):329–39.
25. Koga A, Tokuhashi Y, Ohkawa A, Nishimura T, Takayama K, Ryu J. Effects of Fibronectin on Osteoinductive Capability of Fresh Iliac Bone Marrow Aspirate in Posterolateral Spinal Fusion in Rabbits. Spine (Phila Pa 1976). 2008 May 20;33(12):1318–23.
26. Smiler D, Soltan M, Albitar M. Toward the Identification of Mesenchymal Stem Cells in Bone Marrow and Peripheral Blood for Bone Regeneration. Implant Dent. 2008 Sep;17(3):236–47.
27. Khojasteh A, Behnia H, Hosseini FS, Dehghan MM, Abbasnia P, Abbas FM. The effect of PCL-TCP scaffold loaded with mesenchymal stem cells on vertical bone augmentation in dog mandible: A preliminary report. J Biomed Mater Res Part B Appl Biomater. 2013 Jul;101B (5):848–54.
28. Kadiyala S, Jaiswal N, Bruder SP. Culture-Expanded, Bone Marrow-Derived Mesenchymal Stem Cells Can Regenerate a Critical-Sized Segmental Bone Defect. Tissue Eng. Mary Ann Liebert, Inc. 2 Madison Avenue Larchmont, NY 10538 USA; 1997 Jun 13;3(2):173–85.
29. Niemeyer P, Fechner K, Milz S, Richter W, Suedkamp NP, Mehlhorn AT, et al. Comparison of mesenchymal stem cells from bone marrow and adipose tissue for bone regeneration in a critical size defect of the sheep tibia and the influence of platelet-rich plasma. Biomaterials. 2010 May;31(13):3572–9.
30. Kargozar S, Hashemian SJ, Soleimani M, Milan PB, Askari M, Khalaj V, et al. Acceleration of bone regeneration in bioactive glass/gelatin composite scaffolds seeded with bone marrow-derived mesenchymal stem cells over-expressing bone morphogenetic protein-7. Mater Sci Eng C. 2017 Jun 1; 75:688–98.
31. Nagata MJH, Santinoni CS, Pola NM, de Campos N, Messora MR, Bomfim SRM, et al. Bone marrow aspirate combined with low-level laser therapy: A new therapeutic approach to enhance bone healing. J Photochem Photobiol B Biol. 2013 Apr 5; 121:6–14.
32. Pelegrine AA, Aloise AC, Zimmermann A, de Mello E Oliveira R, Ferreira LM. Repair of critical-size bone defects using bone marrow stromal cells: a histomorphometric study in rabbit calvaria. Part I: use of fresh bone marrow or bone marrow mononuclear fraction. Clin Oral Implants Res. 2014 May;25(5):567–72.
33. Weitao Y, Kangmei K, Xinjia W, Weili Q. Bone regeneration using an injectable calcium phosphate/autologous iliac crest bone composites for segmental ulnar defects in rabbits. J Mater Sci Mater Med. 2008 Jun 6;19(6):2485–92.

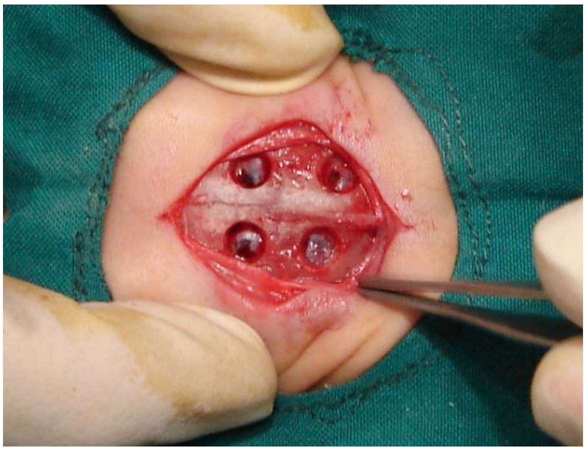

Figure 1. Standardized holes in calvarium of the rabbit.

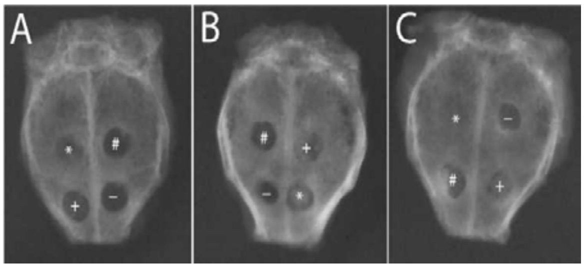

Figure 2. Representative radiographs 4 (A), 8 (B) and 12 (C) weeks after surgery. Cancellous bone is shown as "*", Bioglass-bone Marrow as "+", Bioglass as "#" and "-" is control.

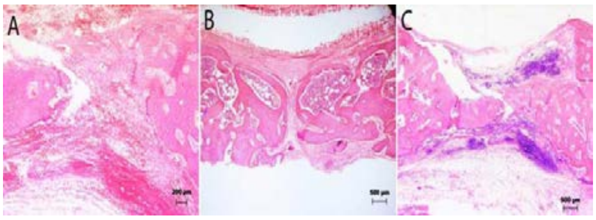

Figure 3. Defect recovery of control after 4 weeks (A), 8 weeks (B) and 12 weeks (C).

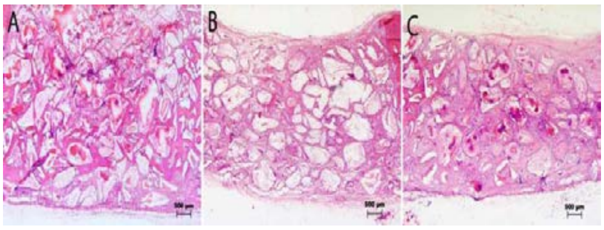

Figure 4. Defect recovery of bioglass treated defects after 4 weeks (A), 8 weeks (B) and 12 weeks(C).

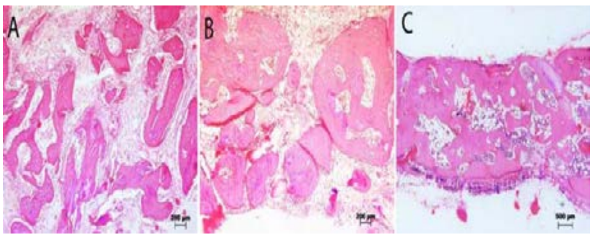

Figure 5. Defect recovery of cancellous bone treated defects after 4 weeks (A), 8 weeks (B) and 12 weeks (C)

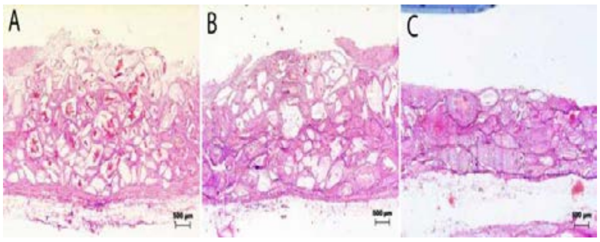

Figure 6. Defect recovery of bioglass-bone marrow treated defects after 4 weeks (A), 8 weeks (B) and 12 weeks (C).

Table 1: Quantified data of radiological observations

Time after treatment	4 Week				8 Week				12 Week			
Treatments	C	CB	B	BBM	C	CB	B	BBM	C	CB	B	BBM
Hole filling	1^{d}	2^{a}	1.3^{c}	1.6^{b}	1.3^{c}	2.6^{a}	1.3^{c}	2^{b}	1.6^{d}	3^{a}	2^{c}	2.3^{b}
Unity of treated area	0^{c}	0^{c}	1.3^{b}	1.6^{a}	0^{c}	0^{c}	1.6^{b}	2^{a}	0^{c}	0^{c}	2^{b}	2.3^{a}
Opacity	1^{d}	2.3^{a}	1.3^{c}	1.6^{b}	1.3^{d}	2.6^{a}	1.6^{c}	2.3^{b}	1.6^{d}	3^{a}	2^{c}	2.6^{b}

C: Control, CB: Cancellous Bone treatment, B: Bioglass treatment, BBM: Bioglass and Bone Marrow treatment. Subscripted alphabet showed significant differences those outcomes of One-way ANOVA in SPSS 16, $P < 0.05$

Table 2: effect of treatments on bone regeneration percentage

	CB	BBM	B	C
After 4 Weeks	52.74 ± 4.45^{a}	29.83 ± 5.63^{b}	22.35 ± 2.67^{c}	10.69 ± 3.13^{d}
After 8 Weeks	69.28 ± 6.20^{a}	49.25 ± 4.71^{b}	33.37 ± 5.23^{c}	22.62 ± 2.39^{d}
After 12 Weeks	89.36 ± 3.38^{a}	74.97 ± 5.22^{b}	56.81 ± 3.95^{c}	31.49 ± 4.15^{d}

CB: Cancellous Bone treatment, BM: Bioglass- Bone Marrow treatment, B: Bioglass treatment, C: Control. Data are average of bone regeneration percentage ± SD. Subscripted alphabet showed significant differences those outcomes of One-way ANOVA in SPSS 16, $P < 0.05$