Indian Journal of Fundamental and Applied Life Sciences ISSN: 2231– 6345 (Online)
An Open Access, Online International Journal Available at http://www.cibtech.org/jls.htm
2015 Vol. 5 (1) January-March, pp. 286-292/Mousavi
Research Article

ALLOGENEIC AND XENOGENEIC BONE MATRIX GELATIN (BMG) EFFECTS ON BONE DEFECT HEALING IN RATS

***Ghafour Mousavi**
Department of Clinical Sciences, Tabriz Branch, Islamic Azad University, Tabriz, Iran
**Author for Correspondence*

ABSTRACT
Several methods are used to enhance bone repair and new bone formation. Bone matrix gelatin (BMG) is recently introduced. The purpose of this study was to evaluate allogeneic and xenogeneic effects of bone matrix gelatin on cancellous bone defect healing in rat models. The experiment was conducted on 30 male adult SD rats which were divided into three groups of control and experiments. After induction of general anesthesia, a hole in size of 2×3 mm in diameter and depth was made using a dental bit in the inner aspect of the between condyles of left femur. In control group defect was left untreated. In experiment groups I and II, allogeneic and xenogeneic BMG was used to fill the bone defect. The BMG was prepared as previously described using Urist method. After 45 days all rats were euthanized. The samples were stained by H&E and histopathology and histomorphometry evaluations were performed. In control group, defect seemed to be filled with adipose tissue and in spite of a moderate osteogenic activity and some osteoblasts could already be seen, specially was detectable, attached to the edge of defects. In experimental groups, many osteoblasts groupings, and young bone trabeculas increased in number with bone trabeculas more organized. Bone trabeculas with regulated osteoblast cells and highly osteogenic activity were already seen. Histomorphometric results, observed that allogeneic and xenogeneic BMG has significant effect on bone healing in experimental groups I and II than control group (p=0.000), but it has no significant effect between experiment groups (p>0/05). This study has shown that exposure to allogeneic and xenogeneic BMG stimulated bone formation in defect area of cancellous bone. The osteoinductive effect of BMG derives from the growth factors within BMG and due to these osteoinductive properties of BMG, provides a more rapid regeneration of bone defects and it is a good choice for the healing of cancellous bone defects.

Keywords: *Histopathology, Bone Matrix Gelatin, Bone, Healing, Rats*

INTRODUCTION
Despite many advances created today in orthopedic surgery, bone healing is still a challenging problem. Due to disadvantages of autologous bone grafting, include limited supply, chronic pain, nerve damage, and wound complications, another alternative grafts are used to fill defects (Mousavi and Rezaie, 2011; Mousavi, 2010). Nowadays many types of bone filling materials including allograft, xenograft and synthetic materials have been developed and have played critical roles in bone repair (Mousavi and Rezaie, 2011; Mousavi, 2010). This new therapeutic technology induces bone regeneration by employing various growth factors, osteogenic cells, and biocompatible scaffolds or a combination of these approaches (Giannoudis *et al.*, 2005). Many growth factors have been implied on bone repair process: platelet-derived growth factors derived (PDGFs), vascular endothelium growth factor (VEGF), transforming growth factors α and β (TGF-α and TGF-β), acid and basic fibroblast growth factors (aFGF and bFGF), epidermal growth factor (EGF), insulin-like growth factors I and II (IGF-I and IGF-II), cement-derived growth factor (CGF), parathyroid hormone related proteins (PTHrP), and bone morphogenetic protein 1 to 12 (BMPs 1-12,) (Kobaiashi *et al.*, 2006; Rezaie *et al.*, 2011). Bone matrix gelatin (BMG) contain many of bone constructing factors such as bone morphogenic protein (BMP) which persuades local mesenchymal cells to differentiate into bone forming cells, a process known as osteoinduction (Mousavi and Rezaie, 2011). In allogenic bone matrix gelatin (BMG), 95% of non-collagen proteins which would eliminate antigenic materials is removed, this process include defatting, demineralization and extraction, so it weakly immunogenic and more biocompatible with the host (Yang

et al., 2010). Reports suggest a positive effect of BMG on bone healing. However, BMG be prepared in two ways, including allogeneic and xenogeneic. The aim of this study was to evaluate the effect of allogeneic and xenogeneic bone matrix gelatin (BMG) on the healing of femoral cancellous bone defect in rat models.

MATERIALS AND METHODS

Animals: The experiment was performed on 30 male adult Sprague-Dawley rats, 250–300 gram. Rats were obtained from the central animal laboratory of Tabriz Branch, Islamic Azad University and were housed in colony rooms with 12/12 hr light/dark cycle at 21±2°C for 2 weeks before initiation of the study, fed with laboratory pellet chow and drinking water was given ad libitum. Investigations using experimental animals were conducted in accordance with the internationally accepted principles for laboratory animal use and care, and our ethical committee on animal care approved the protocol. Rats divided into three groups (control and experiments I&II) of 10 animals each, according to the procedure performed.

Preparation of BMG: To produce bone matrix gelatin as allogeneic and xenogeneic was used Urist method (Urist, 1973). For preparation allogeneic BMG five male rats were euthanized. Diaphyseal shafts of humerus, radius, ulna, femur and tibia were collected and isolated from soft tissues and were placed into liquid nitrogen to avoid possible denaturation of proteins. Xenogeneic BMG was prepared from bovine femur, and the femur was placed in liquid nitrogen. Then allogeneic and xenogeneic BMG Was performed as follows:

The bones were removed from liquid nitrogen and periosteum was separate then by bone cutter was divided into pieces of 5 mm. The bones lipid was removed by chloroform/methanol (1:1), and then demineralized in 0.6 NHCL, and in order to extract soluble proteins of bone were used CaCl2 (2.0 M), EDTA (0.5 M), LiCl (0.8 M) and water (55°C), then were crushed into smaller pieces in liquid nitrogen and were kept in -60°C refrigerator.

Surgical procedure: General anesthesia was induced with Ketamine hydrochloride (Ketamine 10%, Alfasan, Woerden-Holland, 50mg/kg) and Xylazine (Xylazin 2%, Alfasan, Worden-Holland, 5mg/kg) intraperitoneally. The left hind limb was routinely prepared for surgery. A 2-cm skin incision was made on the lateral aspect of the distal femoral condyles. The muscle and articular capsule were dissected bluntly to expose the lateral and medial condyles. A confined cancellous defect was drilled in between of lateral and medial condyles using a low-speed dental bit, saline-cooled in a stepwise fashion. A hole in size of 2×3 mm in diameter and depth was made. In control group defects were left empty. In experiment group (I) defects were filled with allogeneic BMG and in experiment group (II) defects were filled with xenogeneic BMG. Tissue was closed in layers. Animals were monitored postoperatively, and then returned to their cages. Animals received intramuscular injections of Penicillin G, 60000 Iu/kg immediately after surgery and 24 h later, and orally Celecoxib 6 mg/kg for 3 days after surgery.

Histopathology and histomorphometry evaluation: Animals were euthanized after 45 days postoperatively under general anesthesia, with an injection of over dosage of Thiopental sodium causing a quick and painless death. The distal of left femurs include osseous defect were harvested, stripped of soft tissues, and fixed in 10% neutral buffered formalin during five days, for fixation; then dehydrated in 10% EDTA. Finally, they were embedded in paraffin. Serial sections were cut and stained with Haematoxylin and Eosin (H&E) method and used for light microscopic examination under a Nikon microscope (ECLIPSE E200, Japan) to histopathology and histomorphometry evaluation. Histomorphometry analysis were performed by linear measurements through Intersection Latticed Lines using an ocular Latticed Lens comprising 100 cross points to determine the percentage of the defect that was occupied by 1) connective tissue, 2) bone marrow, 3) woven bone and 4) lamellar bone. Thus, at a magnification of 40×, the various components were identified using a mouse cursor (Rezaie *et al.,* 2011; Carmagnola *et al.,* 2002). Bone marrow was identified as a tissue that included adipocites, while connective tissue was defined by the presence of fibroblasts and collagen fibers. Morphometrical analysis was performed also of right health femur, and the normal percentage of lamellar bone, woven bone and bone marrow was assessed.

Statistical analysis: Statistical evaluation of data was performed using the software package SPSS 18 (SPSS Inc., Chicago, IL). Data are reported as mean±standard deviations (SD) for each group. Statistical differences between groups were evaluated with ANOVA followed by the Tukey test to analyze histomorphometric data among groups. The significant level was set at $p<0.05$.

RESULTS AND DISCUSSION

Results

Histopathologically results obtained in the control group, indicating that bone defect is filled by granulation tissue.

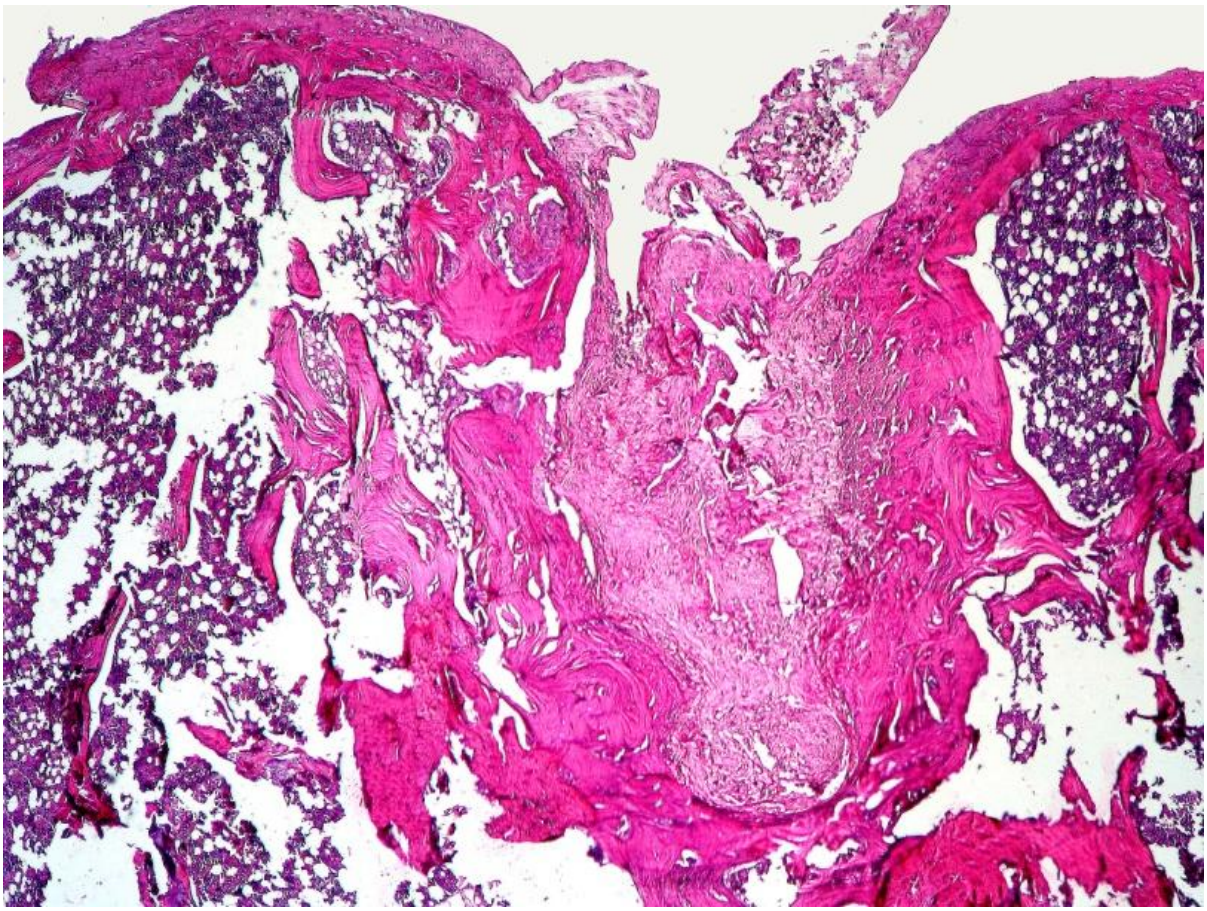

Figure 1: Microscopic section from the healing site in control group shows the defect to be filled with mature granulation tissue. Lateral aspects and deeper portion of the defect is lined with newly formed trabecular bone which is extended from the lateral aspects (spongy trabecular bone) of the defect. There is some detached debris in upper portion of the defect which harbors large numbers of inflammatory cells and necrotic materials (H&E, ×40)

Trabecular newly bone formed in lateral aspects and deeper portion of the defect. Newly formed trabecular bone which is extended from spongy trabecular bone. There are some inflammatory cells and necrotic materials (Figure 1). In Studies microscopic samples obtained from the experiment group (I) that by allogeneic BMG was filled shows that defect filled with well-organized granulation tissue and much of defect has been filled. Articular surface is forming and substitution of fibrous tissue with chondroid tissue. Osteoid, deposition and calcification has lead to smaller improvement in bone formation. Also in this group, lateral aspects and deeper portion of the defect is lined with newly formed trabecular bone which is extended from spongy trabecular bone. Large amount of bone regeneration in the area suggest it

is in a high rebuilding process (Figure 2). In Studies microscopic samples obtained from the experiment group (II) that by xenogeneic BMG was filled shows that well matured granulation tissue is present in apical portion of the defect. The samples of this group shows large amount of bone regeneration in the defect due to rebuilding process. Bone regeneration and bone compact is visible in deep structure of repair site. Lateral and deeper portion of the defect is lined with newly formed trabecular bone which is extended from the lateral aspects (spongy trabecular bone) of the defect (Figure 3). Histopathologic evaluation between two experimental groups, not significant differences were observed. Histomorphometry data obtained indicating that lamellar bone formed in experimental group I&II was significantly more than control group and less than normal bone (p=0.000). Amount of immature bone, bone marrow and connective tissue in experimental groups significantly lower than control group, respectively (p<0/05). In terms of the amount of lamellar bone formation, immature bone, bone marrow and connective tissue in comparisons between the experimental groups studied there was no significant difference (p>0/05) (Table 1).

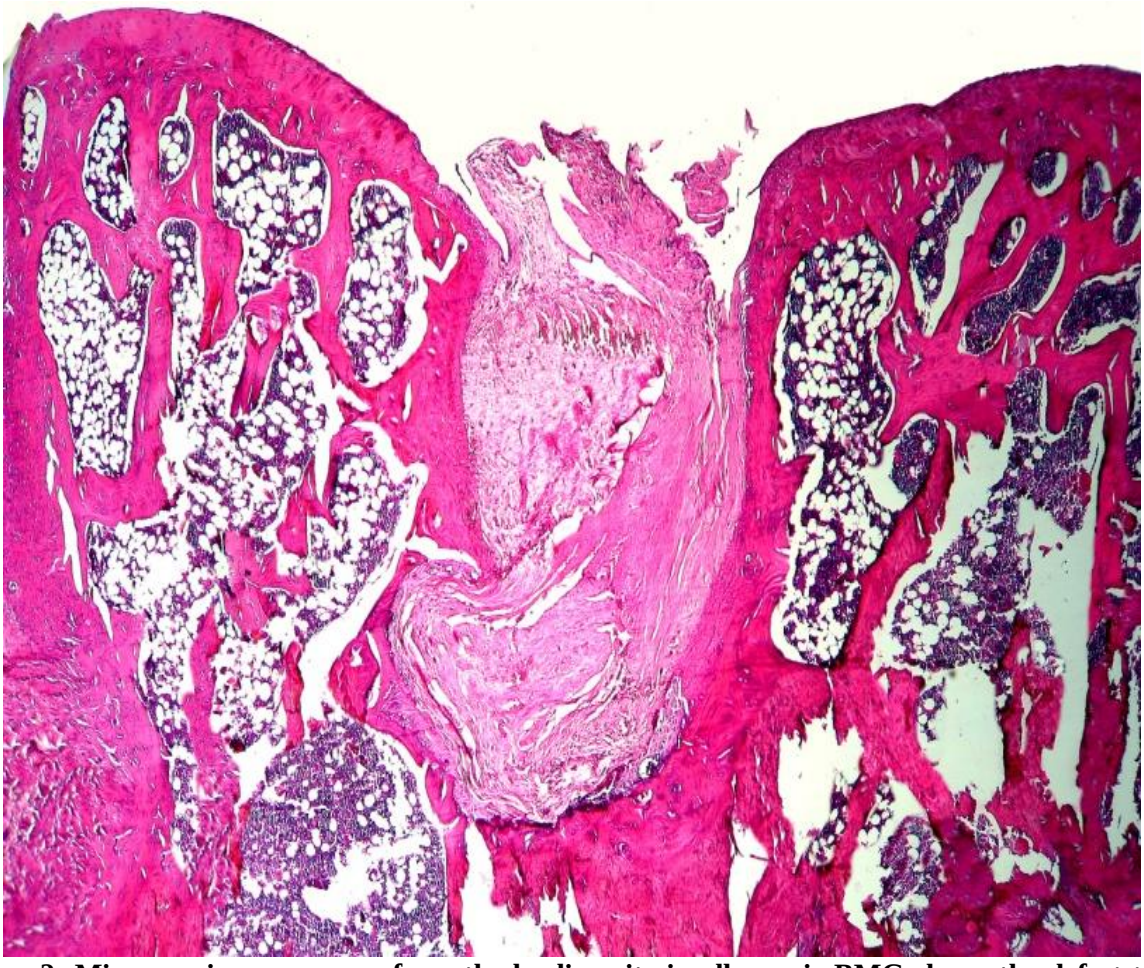

Figure 2: Microscopic appearance from the healing site in allogeneic BMG shows the defect to be filled with well-organized granulation tissue. Substitution of fibrous tissue with chondroid tissue is seen in different parts of repaired construct. Imperfect organic bone matrix, the osteoid, deposition and calcification has lead to smaller improvement in bone formation. A thick layer of newly formed compact bone is prominent in left lateral portion of repaired construct. This newly formed bone because of vital deposition and calcification of osteoid is more acidophilic. Lateral aspects and deeper portion of the defect is lined with newly formed trabecular bone which is extended from the lateral aspects (spongy trabecular bone) of the defect. (H&E, ×40)

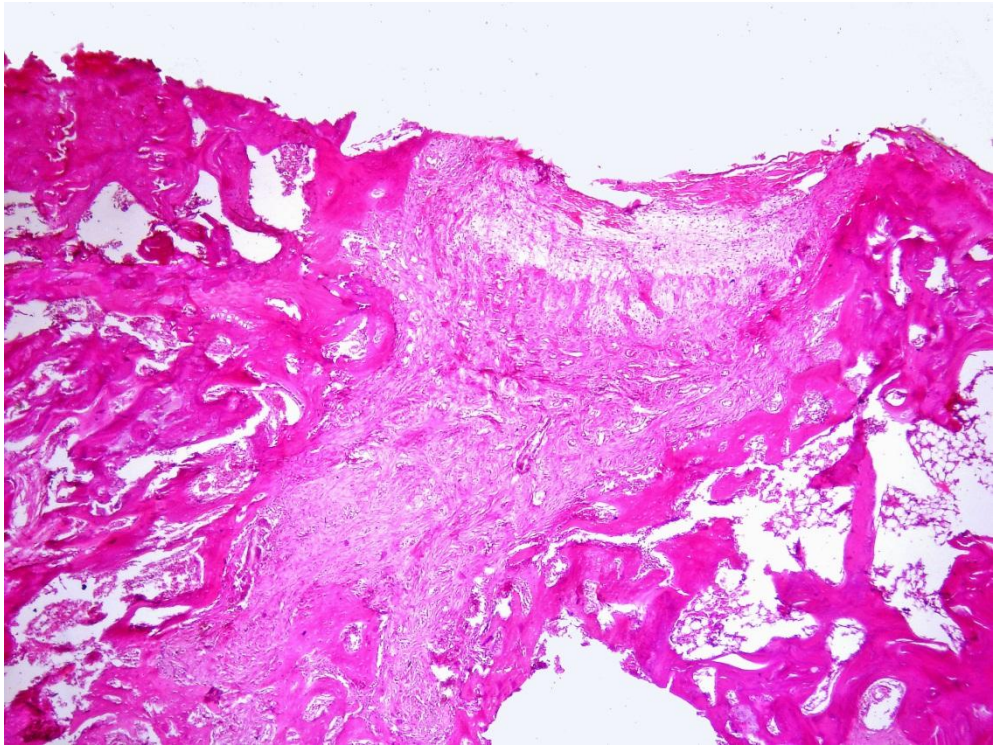

Figure 3: Microscopic appearance from the healing site in xenogeneic BMG shows well matured granulation tissue is present in apical portion of the defect. Remodeling and consolidation of newly formed bone is seen in upper parts of the repaired construct. Organic matrix of bone (osteoid) has been created at the site of repair. It is partially calcified and formed, leading to progressive bone. Mass of newly formed bone is visible in deep structure of repair site. New bone formation due to deposition and calcification of osteoid is strongly eosinophilic. Lateral aspects and deeper portion of the defect is lined with newly formed trabecular bone which is extended from the lateral aspects (spongy trabecular bone) of the defect (H&E, ×40)

Table 1: Comparison of mean and standard deviation of the bone tissue healing area components between the groups studied (10 rats in each)

	Normal bone	Control group	Experimental group I	Experimental group II
Lamellar bone	55.31 ± 3.45^{a}	6.5 ± 0.92^{b}	36.72 ± 0.91^{c}	37.14 ± 1.32^{c}
Immature bone	5 ± 0.75^{a}	30.87 ± 1.45^{b}	27.12 ± 2.13^{c}	29.03 ± 1.14^{c}
Bone marrow	39.69 ± 1.72^{a}	25.5 ± 2.10^{b}	9 ± 1.23^{c}	9.14 ± 2.5^{c}
Connective tissue	0^{a}	37.13^{b}	27.16 ± 2.32^{c}	24.69 ± 1.45^{c}

a,b,c: Dissimilar letters indicate significant differences in each row

DISCUSSION

Bone defect in control group that was empty, due to the absence of osteoinduction or osteoconduction substance, exchange of connective tissue to bone and bone healing process of the extent and intensity was not high. In control group newly formed trabecular bone which is extended from the lateral aspects of spongy trabecular bone of the defect. Control group had a lot of connective tissue and mature granulation tissue in the defect, while the amount of fibrous tissue in the experimental groups were much lower and rather large amount of undifferentiated mesenchyme tissue, there was bone union; and varying amounts of bone had seen that had filled the defect area. In experimental groups I and II, woven bones were less and remodeled to lamellar. Young bone trabeculas incressed in number and were more evident, new

calcified bone matrix and bone neoformation was more compact, with bone trabeculas more organized and calcified. Bone trabeculas outlined medullary spaces filled by abundant hematogenic tissue, similar to normal bone. Assessment of histomorphometry results, show that the majority of the defect in control group was filled by immature bone and connective tissue and significantly more than experimental groups and healthy bone, the amount of lamellar bone in control group is very low, however, the lamellar bone in experimental groups was significantly higher than control group.

The result of this study does not show the difference between the two types of allogeneic and xenogeneic BMG. The results obtained in this study show that BMG causes increases bone induction and accelerate osteogenesis. The principal element of BMG was bone morphogenetic protein (BMP) (Nilsson *et al.,* 1986). BMPs play a role in the differentiation, proliferation, growth inhibition and arrest of maturation of a wide variety of cells, depending on the cellular microenvironment and the interactions with other regulatory factors (Sykaras and Opperman, 2003).

BMPs play an important role in the process of bone modeling and remodeling. The morphogenetic activity of bone matrix is apparent only after its demineralization, which occurs with the controlled action of osteoclasts.

Insulin-like growth factors (IGF-I, IGF-II), TGFβ-1, TGFβ-2, PDGF, basic and acidic fibroblast growth factors, BMPs and other molecules are produced and become incorporated into the forming bone matrix that serves as a reservoir (Sykaras and Opperman, 2003; Mathias *et al.,* 2005; Horisaka *et al.,* 1994). Ohgushi and colleagues showed that, bone morphogenetic protein present in the BMG, causes the cell progressive differentiation to the osteoblast cell and provide more osteogenesis (Ohgushi *et al.,* 1990). Yamashyta and colleagues (1994), this hypothesis suggests that multi-nuclear giant cells are initially present within the lesion has started to absorption BMG, then the activation differentiation of bone cells and remodeling (Yamashita, 1993). Oile studied the role of BMG in human osteoinduction that found satisfactory results (Oile, 1989). Hu in 1993, with cultured human bone matrix gelatinous (hBMG) in rats muscle after 3-4 weeks reported new osteogenesis and bone marrow (Hu, 1993).

Conclusion

The results show that allogeneic and xenogeneic BMG can increase the bone healing in femoral cancellous bone defect in rats and does not show the difference between these two types.

REFERENCES

Carmagnola D, Berglundh T and Lindhev J (2002). The effect of a fibrin glue on the integration of Bio-Oss® with bone tissue. An experimental study in labrador dogs. *Journal of Clinical Periodontology* **29**(5) 377-383.

Giannoudis PV, Dinopoulos H and Tsiridis E (2005). Bone substitutes: an update. *Injury* **36** S20-27.

Horisaka Y, Okamoto Y, Matsumoto N, Yoshimura Y, Hirano A, Nishida M, Kawada J, Yamashit K and Takagi T (1994). Histological changes of implanted collagen material during bone induction. *Journal of Biomedical Materials Research* **28**(1) 97-103.

Hu X (1993). Experimental and clinical investigation of human insoluble bone matrix gelatin. *Clinical Orthopaedics and Related Research* **293** 360-365.

Kobaiashi Wilson EM, Barbieri CH and Mazzer N (2006). Bone healing stimulation by Platelet Rich Autogenous Plasma. An experimental study in rabbits. *Acta Ortopédica Brasileira* **14**(4) 208-212.

Mathias PG, Bostrom MD and Daniel A (2005). Seigerman The Clinical Use of Allografts, Demineralized Bone Matrices, Synthetic Bone Graft Substitutes and Osteoinductive Growth Factors: A Survey Study. *HSS Journal* **1**(1) 9–18.

Mousavi Gh, Sharifi D, Mohajeri D, Rezaie A, Mortazavi P, Soroori S and Hesaraki S (2010). Effect of Calcium Phosphate Bone Cement and Type I Collagen Mixture on Healing of Segmental Bone Defect in Rabbit Radius. *Australian Journal of Basic and Applied Sciences* **4** 5144-5153.

Mousavi Gh and Rezaie A (2011). Biomechanical effects of calcium phosphate bone cement and bone matrix gelatin mixture on healing of bone defect in rabbits. *World Applied Sciences Journal* **13**(9) 2042-2046.

Nilsson OS, Urist MR, Dawson EG, Schmalzried TP and Finerman GA (1986). Bone repair induced by bone morphogenetic protein in ulnar defects in dogs. *Journal of Bone and Joint Surgery British* **68**(4) 635-642.

Ohgushi H, Okumura M, Tamai S, Shors EC and Caplan AI (1990). Marrow cell induced osteogenesis in porous hydroxyapatite and tricalcium phosphate: A comparative histomorphometric study of ectopic bone formation. *Journal of Biomedical Materials Research* **24**(12) 1563-1570.

Oile S (1989). Bone repair induced by bone morphogemetic protein in ulnar defects in dogs. *Journal of Bone and Joint Surgery* **68**(4) 635-642.

Rezaie A, Mousavi Gh, Mohajeri D and Gabelsardrodi V (2011). Effect of autogenous Platelet-rich Plasma (PRP) on femoral cancellous bone defect healing in Alloxan-induced diabetic rabbits. *Australian Journal of Basic and Applied Sciences* **5** 800-808.

Sykaras N and Opperman LA (2003). Bone morphogenetic proteins (BMPs): how do they function and what can they offer the clinician? *Journal of Oral Science* **45**(2) 57-73.

Urist MR (1973). Bone Morphogenesis in implants of insoluble bone gelatin. *Proceedings of the National Academy of Sciences of the United States of America* **70**(12 pt1-2) 3511-3515.

Yamashita K (1993). Ultrastructure of calcified muscle fibers at the implantation site of dematerialized bone matrix gelatin. *International Journal of Experimental Pathology* **74**(6) 547-552.

Yang B, Yin Z, Cao J, Shi Z, Zhang Z, Song H, Liu F and Caterson B (2010). In vitro cartilage tissue engineering using cancellous bone matrix gelatin as a biodegradable scaffold. *Biomedical Materials* **5**(4) 045003.