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Biomechanical Effects of Calcium Phosphate Bone Cement and Bone Matrix Gelatin Mixture on Healing of Bone Defect in Rabbits

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Abstract: Several methods are used to enhance bone repair and new bone formation. Bone matrix gelatin (BMG) is recently introduced. The objective of this study was to evaluate of biomechanical effects of a mixture of calcium phosphate cement and BMG on bone repair in rabbit models. In this study, 45 male rabbits, were used and divided into three groups of 15 animals each. 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 control group defect was left untreated. In group 2, calcium phosphate cement was used to fill the bone defect and in group 3, a mixture of calcium phosphate cement and BMG was used to fill the bone defect. Five rabbits of each group were euthanized at 1, 2 and 3 months intervals for evaluation of biomechanically changes. Results show that the mean load for fracturing in group 3 after 1, 2 and 3 months of surgery, is significantly higher than group 1 and group 2 ($p<0/001$). Biomechanical results indicate that most mean load for fracturing is related to healthy bone (373/7±3/55) and mean load in group 3, after three month of surgery (360/2±1/65) is extent to healthy bone. Comparisons between the study groups in 1, 2 and 3 months after surgery was showed significant changes between the group 2 and control group ($p<0/001$). A comparison evaluation between the groups 2 and 3 was showed significant changes after 1, 2 and 3 months of surgery ($p<0/001$). In this study calcium phosphate bone cement and bone matrix gelatin mixture is intended to be suitable for defects of any shape; this mixture is the osteoinductive property which provides better strength to the healing site.

Key words: Biomechanical · Calcium phosphate cement · Bone matrix gelatin · Bone healing · Rabbits

INTRODUCTION

The treatment of massive bone defects is the most challenging problem; many researchers are trying to find materials or drugs that can improve bone healing [1, 2]. Autogenous bone, typically from the iliac crest, remains the preferred source of bone for grafting. However, disadvantages of autologous bone grafting that include limited supply, chronic pain, nerve damage, and wound complications [3-5]. Another alternative grafts to fill defects, including allograft and synthetic materials. But use of allograft has many problems is includeding potential disease transmission, histoincompatibility, and possibly lower union rates [4]. Therefore, bone tissue engineering has been attracting much attention, nowadays; bone tissue engineering is one of the most important roles in medical science research. Many types of bone filling materials such as different types of calcium phosphate bone cement have been developed and have played critical roles in bone repair [3]. Calcium phosphate bone cement used as an alternative bone substitute in bone grafting [6]. It has been reported that calcium phosphate bone cement has excellent osteoconduction and resorbability when filling the bone defect [7-10], Physical and chemical characteristics of these materials are very similar to natural minerals of bone [11], and they lack the disadvantage of autografts and allograft [4]. In allogenic bone matrix gelatin (BMG), 95% of non-collagen proteins which would eliminate antigenicmaterials is removed, this process include defatting, demineralization and extraction, so it weakly immunogenic and more biocompatible with the host [12]. 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 [13]. Many authors have confirmed the successful defect reconstruction using demineralized

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bone matrix (DBM) or bone matrix gelatin (BMG) [14]. To the best of our knowledge, the use of calcium phosphate bone cement and bone matrix gelatine to combine together can play an important role in bone healing. So in this study, we evaluated the biomechanical effect of a mixture of calcium phosphate bone cement and bone matrix gelatine on the healing of radial cortical bone defect in rabbit models.

MATERIALS AND METHODS

Animals: Investigations using experimental animals were conducted in accordance with the internationally accepted principles for laboratory animal use and care as found in the United States guidelines (United States National Institutes for Health publication no. 85-23, revised in 1985), and our ethical committee on animal care approved the protocol. 45 male adult New Zealand white rabbits, 2.5-3kg were used. Rabbits were obtained from the central animal laboratory of Islamic Azad University-Tabriz Branch 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. Rabbits divided into three groups of 15 animals each, according to the procedure performed.

Calcium Phosphate Bone Cement: Cement used in this study consists of powder contained calcium and phosphorous based ingredients include Ca4(PO4)2O and CaHPO4 and the liquid part is distilled water containing Polyacrylic Acid and 2-Hydroxyethyl Methacrylate (HEMA) and 2,2,4-Trimethyl Hexamethylene Dicarbonate.

Preparation of Bone Matrix Gelatine: To produce bone matrix gelatin was used Urist method [15, 16]. Five male rabbits 2-2.5kg 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. The bone was 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 $CaCl_2$ (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 an IV injection of Ketamine hydrochloride (Ketamine 10%, Alfasan, Woerden-Holland, 50mg/kg) and Xylazine (Xylazin 2%, Alfasan, Worden-Holland, 5mg/kg) and right radius was routinely prepared for surgery. A 3-cm longitudinal skin incision was made on the radius. The space between extensor and flexor muscles groups was dissected, providing a wide view of radius, of which periosteum was fully dissected. A segmental bone defect was created in the middle of the radius shaft, 10-mm in length, using a low-speed orthopedic motor saw, saline-cooled in a stepwise fashion. The defect then was washed carefully with a physiological saline solution. In group 1 (control group) defect was left empty. In group 2, the bone defect was filled with calcium phosphate bone cement, for preparation of calcium phosphate bone cement 1 mL of the liquid part was mixed into slurry with 1gr of the powder part. In group 3, the bone defect was filled with a mixture of calcium phosphate bone cement and BMG, for preparation of this mixture, 1 mL of liquid part of bone cement was mixed with 200 mgr of BMG and 1gr of the powder part of bone cement. The muscle attachment was repaired and skin was closed in layers. Intramuscular injection of 0.05 mg/kg dexamethasone (Vetacoid®, Aburaihan Co., Iran) and 40000 IU/kg Penicillin G, Benzedrine, Procaine and Potassium 2:1:1 (Nasr Fariman Co., Iran) was performed.

Biomechanical Analysis: Five rabbits were euthanized with an intravenous injection of an over dosage of thiopental sodium, causing a quick and painless death, at 1, 2 and 3 postoperative month in each groups. Biomechanical analyses were performed also of left radius, and the normal load bearing of health radius was assessed. So the operated right radius and left health radius were harvested, and wrapped in saline-soaked gauze, frozen, and stored. Specimens were thawed at room temperature in a saline bath prior to mechanical testing. All mechanical testing were performed using a Zwick/Roell Z010 with a crosshead speed of 0.01 mm/sec. A load-distance curve was recorded to determine mechanical properties. Load bearing was obtained with maximum load recorded of the linear portion of the load-distance curve.

Statistical Analysis: Statistical evaluation of data was performed using the software package SPSS version 13 (SPSS Inc., Chicago, IL). Data are reported as mean±standard deviations (SD). The significant level was set at $p<0.05$. Statistical comparisons were used analysis of variance (ANOVA). Tukey HSD multiple comparison testing was used to determine experimental defects with normal bone.

Table 1: The mean±SD of load bearing in study groups (N/mm). (Number of rabbits in each group and each month, is the fifth)

	1 month after surgery	2 month after surgery	3 month after surgery
Healthy bone	373/7±3/55[a]	373/7±3/55[a]	373/7±3/55[a]
Group1 (control)	68/2±1/42[b]	80/6±1/32[b]	94/4±1/16[b]
Group2 (bone cement)	162±1/94C	204±1/58C	288/6±4/09c
Group3 (bone cement+BMG)	200/6±1/16[d]	280/4±3/32[d]	360/2±1/65[d]

a b c d: Dissimilar letters is indicate significant differences of each column

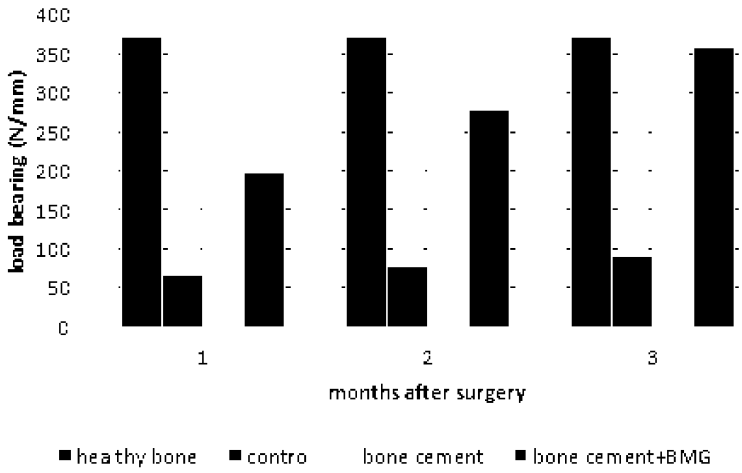

Fig. 1: Comparison of mean load bearing in study groups, after 1, 2 and 3 month of surgery

RESULTS

Results of mechanical tests obtained in this study show that the mean load for fracturing in group 3 (mixture of bone cement and BMG) after 1, 2 and 3 months of surgery, is significantly higher than group 1 (control) and group 2 (bone cement) ($p<0/001$). Biomechanical results indicate that most mean load for fracturing is related to healthy bone and mean load in group 3, after three month of surgery is extent to healthy bone, however, statistical evaluation showed significant changes in this group after three months of surgery to healthy bones ($p<0/021$). Comparisons between the study groups in 1, 2 and 3 months after surgery was showed significant changes between the group 2 and control group ($p<0/001$). A comparison evaluation between the groups 2 and 3 was showed significant changes after 1, 2 and 3 months of surgery ($p<0/001$). Comparisons results of group 3 with control group after 1, 2 and 3 months of surgery was showed a significant changes ($p<0/001$). Also compare pairs of studied groups with healthy bone showed significant changes during the period was studied ($p<0/001$). The mean±SD of mechanical test results for each group are provided in table 1 and figure 1 show mean load for fracturing in study groups after 1, 2 and 3 months of surgery.

DISCUSSION

An ideal bone graft substitute should have osteoconductive, osteoinductive and osteogenic properties [3]. As a result, some investigators used mixtures of synthetic biomaterials and osteoinductive organic agents to achieve better results [17]. The current study aimed to evaluate the positive effect of calcium phosphate bone cement mixed with bone matrix gelatine on bone response in comparison of load bearing to determine the regional biomechanical properties of the radius defect in rabbit. In our study, rabbits were divided into three groups, and each group were divided into three subgroups as 1, 2 and 3 months after surgery to demonstrate the mechanical properties of the radius in each group per time. The highest mechanical results were obtained in healthy bone and group of calcium phosphate bone cement and bone matrix gelatine mixture, 3 month after surgery. Furthermore, the lowest results were obtained in control group that defect was empty (Table 1). It seems that mixing both bone matrix gelatine and bone cement, due to its high osteoinductive, become stronger on load bearing as the remodeling process continues which were near the value of intact bone after three month. The bone cement can be shaped into any complicated defect and filled into any intricate cavity, it can adapt to the bone defect and providing a good

fixation and appropriate contact necessary for stimulating bone ingrowth [3, 18, 19]. In this study also calcium phosphate bone cement and bone matrix gelatine mixture is intended to be suitable for defects of any shape. Bone cement is replaced by new bone, as the healing process progresses, this is the osteoconductive property which provides better strength to the healing site [20]. The BMG preparation process, as previously observed by Urist et al [16, 21]. The principal element of BMG was bone morphogenetic protein (BMP). 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 nteractions with other regulatory factors [22]. 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), TGFâ-1, TGFâ-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 [22].

In conclusion, the current study successfully constructed radial cortical bone defect using allogeneic BMG and calcium phosphate bone cement mixture as the biomaterials. This mixture can be successfully used for bone healing due to good biocompatibility, high mechanical strength and weak immunogenicity.

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