Comparison of Polyglycolactic - Hydroxyapatite - CHITOSAN Scaffolds with or without Mesenchymal Stem Cells in an In-vivo Study

Ali Yeganeh[1], Babak Athari[1], Nader Tavakoli[2], Mehdi Moghtadaei[1*]

[1] Trauma and Injury Research Center, Shafa Orthopedic Hospital, Iran University of Medical Science, Tehran, Iran.
[2]Trauma and Injury Research Center, Department of Emergency Medicine, Iran University of Medical Science, Tehran, Iran.

ABSTRACT

Fractal bone fracture frequently refers to defects of critical size due to increased severity of musculoskeletal trauma, tumor removal, fracture of the bone and recurrent arthroplasty surgery of the joints, and lack of self-repair, which are frequently occurred in orthopedic. Many studies focused on mesenchymal stem cells (MSCs) for the repairment of bone fractures and defects. In this regard, we tried to use this to carry out the exact effects of this scaffold on bone defect repair with bone marrow mesenchymal cells in vivo. Thirty rats with a bone defect of 7 mm placed under the standard conditions for this work. Also, they evaluated for the imaging studies (CT scan) under anesthesia. In the only scaffold group, the findings were greatly similar to the previous one (scaffold with MSCs) after 12 weeks. In the group without scaffold, a new bone was not formed between the lesions and only a small amount of new bone was formed at the ends of the femur. After 12 weeks, there was no evidence of scaffold in histological studies. In the scaffold with MSCs, the thickness and bulk volume of the lamellar were higher in comparison to the only scaffold group. Also in histological study, the accumulation of inflammatory cells has not seen in any of the specimens. In conclusion, the scaffold was used in this study can improve the activity of MSCs in both histological and radiological studies. Also, in the scaffold with MSCs, the thickness and bulk volume of the lamellar were higher in comparison to the only scaffold-treated group.

Key Words: Mesenchymal Stem Cells, Scaffolds, Hydroxyapatite, Chitosan, Polyglycolactic

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INTRODUCTION

It has estimated that 5 to 10% of fractures, progress to a not-well or not-proper repairmen status [1]. Fractal bone fracture frequently refers to defects of critical size due to increased severity of musculoskeletal trauma, tumor removal, fracture of the bone and recurrent arthroplasty surgery of the joints, and lack of self-repair, which are frequently occurred in orthopedic. The total amount cost of musculoskeletal disorders estimated to $ 215 billion annually [2].

Recently, formation a bone that exactly is in accordance with tissue engineering is an ideal choice to rebuild fractures and the limitations of autograft transplantation. Components required for tissue engineering processes include cells, scaffolds, and growth factors. In this regard, many studies preferred to use mesenchymal stem cells (MSCs) for bone repairmen [3-10].

Two characteristics identify of MSCs include, the ability of proliferation and differentiate to multiple cell lines. These cells are presented in many tissues of adults, including bone marrow [11-13]. Also, MSCs have a property of immune suppression, which is shown in both in vitro [14-16] and in vivo [16-20]. Studies have shown that MSCS could not express any type of HLA in Class II, this condition indicates that transplantation might not initiate the immune response of reception [21]. It has been proven that these cells could not express the stimulatory molecules of CD40, CD80, and CD81, which potentially results in anergy of T-cells; in addition, MSCs have an

Corresponding author: Mehdi Moghtadaei
Address: Bone and Joint Reconstruction Research Center, Shafa Orthopedic Hospital, Iran University of Medical Science, Tehran, Iran.
E-mail: ✉ mmoghtadaei@gmail.com
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ability to inhibit the proliferation of T-cells through various mechanisms [16, 22]. MSCs could decrease the differentiation of monocyte and CD34 precursor cells into dendritic cells (DCs) [14]. On the other hand, these cells can inhibit the proliferation of B-cells and also inhibit the releasing of cytokines and cellular cytotoxicities which are related to the natural killer cells (NK-cells) [19]. For this reason, using of MSCs has been suggested in many papers for transplantation of bone, heart, spleen, and etc. [20, 23-25]

Scaffolds have the similar properties to the biological and mechanical properties of a real tissue matrices. Scaffolds could create a three-dimensional (3-D) space for the cells during growth and differentiation. In this 3-D patterns, the growth, migration, differentiation, and viability of patients' cells are stimulated by this scaffolds [26, 27]. Also, scaffolds act as a carrier for transferring of cells and motivate some factors to the site of the lesion to repair faster. [28]

Recently, a new combination of Poly Glycolic Lactic Acid (PLGA), CHITOSAN and Hydroxyapatite (HA) was designed to produce more robustness, mechanical characteristic, destructive options, and microstructural properties in comparison to other existing polymers. In this regard, we tried to use this to carry out the exact effects of this scaffold on bone defect repair with bone marrow mesenchymal cells in vivo.

MATERIALS AND METHODS

a. Preparation of MSCs and scaffolds

Patients referring to Rasool Akram Hospital with a complaint of traumatic fracture were talkedto get their satisfaction and also examination were done for the presence of HIV, HBV, HCV infections of bone marrow specimens. Also, the samples were obtained from the pelvic bone under general anesthesia. The samples enriched by using Density Gradient Centrifugation Ficoll method from mononuclear cells and then transferred to LDMEM and FBS 10% for culture. To perform a cell passage, first remove the old culture medium and wash the cells with 2-3 ml of PBS. Then, with the aid of sterile sampler, a milliliter of trypsin solution of 0.25% injected in the flasks and put them into the incubator for about 2-3 min at 37 degree of centigrade. After transferring the volume of the flasks to 6 ml, the contents were transferred to the tube of Falcon and then centrifuged at 800 rpm for 5 minutes.

Cell differentiation is performed solely for the characterization of mesenchymal cells, and in this project, undifferentiated cells linked with the scaffold. At first step, in senary plates, we cultured 50,000 and replaced their culture medium every 3 to 4 days until that all cells will be confluent. Afterward, in 4 chambers, the differentiative media was added (osteogenic or adipogenic) and replaced every 3 to 4 days until the morphogenesis of the cells towards osteoblasts and adipocyte.

The fabrication method of PCL scaffold was previously reported [29]. Briefly, several requirements should be considered in the design of 3D scaffolds for bone tissue engineering [30-32]. First of all, an ideal bone scaffold should have sufficient porosity to accommodate osteoblasts or osteoprogenitor cells, to support cell proliferation and differentiation, and to enhance bone tissue formation. High porosity (such as ¸90%) is necessary for scaffolds for any tissue engineering applications, including bone [33, 34]. High interconnectivity between pores are also desirable for uniform cell seeding and distribution, the diffusion of nutrients to and metabolites out from the cell/scaffold constructs. A variety of processing technologies has been developed to fabricate porous 3D polymeric scaffolds for bone regeneration. These techniques mainly include solvent casting and particulate leaching, gas foaming, emulsion freeze-drying, electrospinning, rapid prototyping, and thermally induced phase separation.

Cells were seeded at 50,000 cells/well onto both 24-wellplate and scaffold (2 mm in height and 14 mm. in diameter, cells seeding ratio 1:2). Cell attachment was performed by a 3–(4,5-dimethylthiazol-2-yl)22,5-diphenyltetrazoliumbromide (MTT)(USB Corporation, USA) assay at 24, 48, and 72 h, respectively. The experiment was performed in triplicate. Briefly, cells were incubated with 0.5 mg mL-1 MTT in DMEM at 37 degrees of Centigrade for 45 min. After incubation, the MTT solution was removed and a buffer solution containing Dimetylsulfoxide (DMSO) (Sigma, USA) was added to dissolve the formazan crystals. After 10 min of agitation, each sample solution was measured for optical density using microplate reader (XMARK, BIO-RAD, USA) at 570 nm.

b. Animals and conditions

Thirty Wistar rats (300-350 g) were placed under laboratory anesthesia after one week in an experimental animal storage room (received intraperitoneal of ketamine and zeylinin), and after sterilizing the skin, incision on the Femoral site bone was done, In the next step, femoral muscles were discarded and the femur bone was dissected from its muscle connections.

After insertion of the plaque and fixation, a bone defect was made up to approximately 7 mm. All animals placed under the standard conditions of water, food, and light. The following day, under anesthesia, animals were evaluated under imaging studies (CT scan).

Also, the groups which were involved in this study include as follows.

1. The first group (G1 n = 10) with scaffold cover and mesenchymal cells on the bone defect site.
2. The second group (G2 n = 10) with scaffold covering on the site of the bone defect.
3. The third group (G3 n = 10) with bone defect without scaffold and mesenchymal cells.

c. Histological study

After 12 weeks, femoral bone was removed from muscle connections and fixed in 10% formalin solution for 24

hours and placed in 10% EDTA solution for 2 months at room temperature. Finally, samples were examined for bone formation and the presence of inflammatory cells with optical microscopy. H & E staining was used to evaluate the repairment with optical microscopy. Cell infiltration and formation of osteoid were considered as repairment criteria.

d. Statistical analyses

The experiments were performed in three separate setting using three different donors. Data were expressed as mean 6 standard deviation (SD). Statistical analyses were performed using a one-way analysis of variance (ANOVA), followed by Tukey post hoc test for multiple group comparison and Kruskal–Wallis one-way ANOVA. A P values <0.05 was considered to be statistically significant difference.

RESULTS

a. Animal model of bone defect

After the surgery and the creation of experimental bone defect models, no samples were lost during the 3 months of this project. Bone defect was confirmed in the radiological and CT scan studies. Improvement of lesions in the center of the bone was calculated based on the percentage of bone formation in the coronal view of CT scan.

After 12 weeks of the bone grafting, the new formation of bone was formed in the site of the bone defect, the scaffold was completely re-absorbed, replaced by a newly formed bone that was present throughout the lesion. In the scaffold with MSCs group, complete bone defect recovery was observed in all specimens.

In the only scaffold group, the findings were greatly similar to the previous one (scaffold with MSCs) after 12 weeks. In the group without scaffold, a new bone was not formed between the lesions and only a small amount of new bone was formed at the ends of the femur. In CT scan studies, 100 ± 12% of bone defects were filled with newly formed bone, but in the scaffold group only 67 ± 5% of the lesion was filled by new tissue, in the non-scaffold group, only 12 ± 3% of the new bone was around the ends Femur bone was seen at the site of the lesion (Fig. 1-3).

b. Histological results

Animals were evaluated for tissue repairmetns about three months after surgery. Histological studies indicated that the bone formation with normal and typical bone structure at the site of the lesion after about 12 weeks of transplantation in both groups of scaffolds and scaffolds with MSCs. After 12 weeks, there was no evidence of scaffold in histological studies. In the scaffold with MSCs, the thickness and bulk volume of the lamellar were higher in comparison to the only scaffold group. Also in histological study, the accumulation of inflammatory cells has not seen in any of the specimens. The absence of inflammatory cells and tissue repairment indicate the extent of the scaffold biocompatibility (Fig. 4, 5).

DISCUSSION

In this study, we examined the application of bone marrow mesenchymal stem cells along with the new PLGA / HA / CS scaffold. The present scaffold demonstrated that using this scaffold could induce proper rehabilitation through increase cell adhesion, cell proliferation, etc. In our in vivo study, which was performed with bone defect in rat femur, PLGA / HA / CS scaffold alone or in combination with MSCs cells, could improve the lesions, however, in only scaffold experiment, the volume of lesion recovery was lower than the scaffold with MSCs and the injury was not completely recovered.

The Past in vivo studies showed the formation of bone marrow in the lesion site in both only scaffold and scaffolds with cells. Although in the only scaffold group, the bone volume was less and transverse, the lesion was not filled, but in the scaffold group with the cell, the lesion recovered completely, which could indicate the role of MSCs in the new bone formation and differentiation towards the osteoblast cells. Many studies suggest that MSCs can release many growth factors which lead to change in their surroundings and proliferation of precursor cells. In this study, lesion recovery was preformed through two possible mechanisms. First, MSCs were converted directly to osteoblasts, and secondly, MSCs, through secretion of cytokines and growth factors, led to lesion recovery. Further studies should be designed to determine the role of MSCs in improving bone loss in in vivo conditions [35-39].

One of the first polymers, which was used in the field of bone mineral engineering, was the combination of polycarboxylic acid, glycolic acid (PLGA) [40]. Liao et al. were investigated the mineralization effect of hydroxyapatite on the nano-PLGA scaffold; the results indicated its significant effects on the biocompatibility of the scaffold [41]. Park et al. used PLGA-containing scaffolds to accelerate the healing of bone loss in the rabbit's ulna bone. They used a combination of periosteal cells and scaffolds for bone defects. Their study indicated a significant improvement in the bone defect in the scaffold transplant group containing PLGA polymerase and mesenchymal stem cells [42]. Chiu et al. examined the polylactic acid scaffold (PLLA) in terms of cell migration and its penetration, with the result of high biocompatibility and adhesion of the scaffold to the cells [43].

In a study by Jose et al. on PLGA and collagen, the results indicated that the combination of 80/20 of PLGA to collagen was the best structural and mechanical condition for use as a scaffold for bone tissue engineering [44]. Studies have shown that only hydroxyapatite scaffold has no proper mechanical properties, and the formed bone in this scaffold cannot withstand the long-term mechanical constraints for remodeling. [45]. For solving this problem, it has suggested using the degradable polymers. Adding polymers such as PLGA to hydroxyapatite can improve bone defects higher than older scaffolds [28, 46-50].

In another study by Lin and Yoon et al., the combination of mesenchymal stem cells and PLGA scaffolds was used in the rabbit femur. At the end of week 4, bone defect in all

rabbits was fully recovered by CT scan and histological studies [51, 52].

CONCLUSIONS

The scaffold was used in this study can improve the activity of MSCs in both histological and radiological studies. Also, in the scaffold with MSCs, the thickness and bulk volume of the lamellar were higher in comparison to the only scaffold-treated group.

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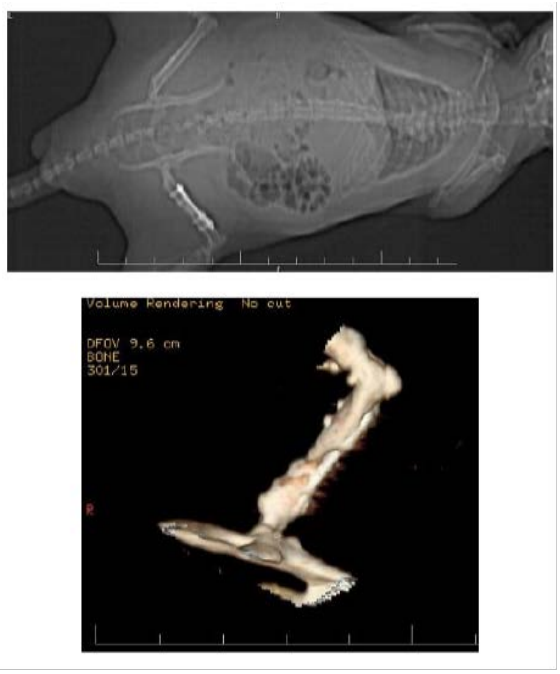

Fig.1. In the non-cellular scaffold group, after 3 months of transplantation, a new bone was formed, but only 67 ± 5% of the lesion was filled with new tissue.

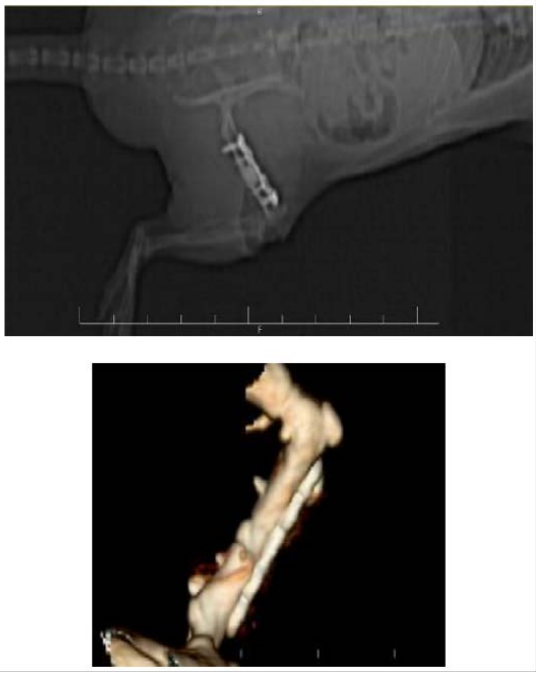

Fig.2. In the scaffold group with bone marrow mesenchymal stem cells, the bone was completely recovered, and the bone mass volume was significantly higher than the volume of bone defect. (100±12).

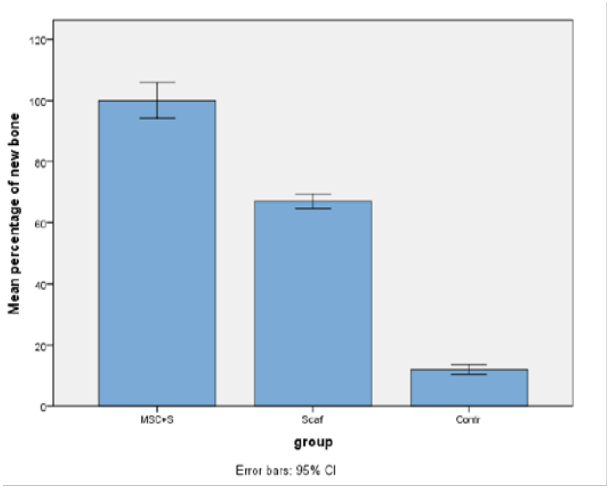

Fig.3. Percentage of bone formation at the site of the lesion. There was a significant difference between all groups (P≥0.05).

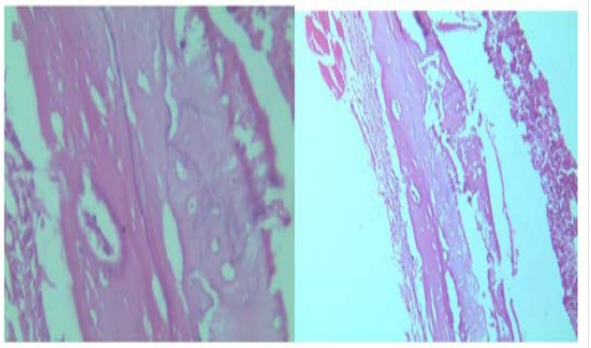

Fig.4. Complete bone formation at the site of the lesion in the only scaffold group. The thickness and volume of the bone formed were lower than the scaffold with cells group. In magnification, inflammatory cells do not appear in the newly formed bone.

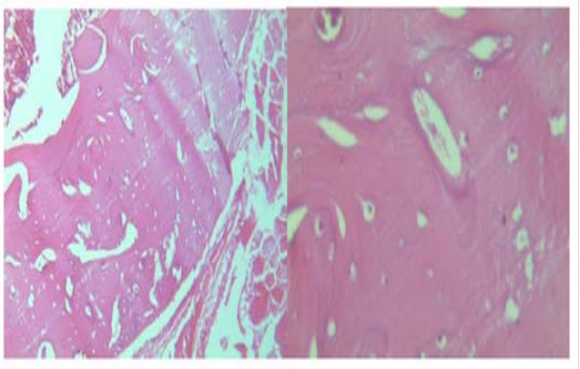

Fig.5. Complete bone formation at the site of the lesion in the scaffold group with mesenchymal stem cells. The thickness and volume of the bone formed were higher than the only scaffold group. In magnification, inflammatory cells do not appear in the newly formed bone.