10.21608/ADJC.2023.220628.1358 Advanced Dental Journal
Volume 5 (2023) | Issue 3 | Pages 470-477

Original Article

Evaluation of Biphasic Calcium Phosphate Combined Platelet-Rich Fibrin on Bone Regeneration in Surgical Created Bone Defect: A Comparative Histological Experimental Study

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Submitted: 02-07-2023
Accepted: 14-07-2023

Abstract

Aim: The aim of this study was to evaluate histologically the effect of biphasic calcium phosphate combined platelet-rich fibrin on bone regeneration in a surgically created bone defect. **Subjects and methods:** Sixteen bony surgical defects were created in tibia bone of male white New Zealand rabbits. According to graft material, the surgical defects were divided into two study groups and one control group that left empty without grafting. Study Group (A) was grafted with BCP+PRF, while Study Group (B) was grafted with BCP only. Decapitation was carried out after one month and the grafted defects were dissected and prepared for histological examination. **Results:** Both study groups showed better healing compared to the control group which was filled with granulation tissue infiltrated by chronic inflammatory cells. Both grafted groups showed lamellar bone formation filling the defect and extending into the surface in some cases. In group (A), bone trabeculae filling the defect were thicker and mostly mineralized, compared to group (B). In addition, cartilage formation was noticed in group (A). **Conclusion:** Biphasic calcium phosphate combined platelet-rich fibrin is a valuable graft material and provide superior quality of bone regeneration and a promising biomaterials in inducing cartilage repair.

Keywords: Biphasic Calcium Phosphate, Platelet-Rich Fibrin, Bone Regeneration, Histological examination

I. INTRODUCTION

Although autogenous bone graft has been used extensively enhance bone formation in different bony defects, but with less than ideal results, due to its drawbacks. [1, 2] The main advantage of using an alternative to autogenous bone graft is the avoidance of a second site surgery. Many studies have been conducted to evaluate various bone substitutes in order to avoid these adverse effects, but there is still controversy whether artificial bone substitutes can be considered an alternative to autogenous graft or not. (3)

Biphasic calcium phosphate (BCP), mixture of hydroxyapatite (HA) and B-Tricalcium phosphate (B-TCP) is a synthetic bone substitute which has the native composition and mechanical features of natural bone. .This biocompatible material which is widely used in orthopaedic, maxillofacial, and periodontal surgery, has mainly osteoconductive properties. [4, 5] Recently platelets preparations have been used to enhance osteoinductive properties due to their contents of multiple growth factors. Therefore, there have been various efforts to improve BCP osteogenic potential with

Platelet-Rich Plasma (PRP). [5] The benefit behind using PRP combined with BCP to enhance bone formation.

Although BCP treated with PRP has shown improved osteoinductive potentials when compared with untreated BCP, there is various disadvantages which limit the use of PRP, such as liability of infection transmission due to using bovine thrombin plus it is time-consuming and technique sensitive [6]

Platelet-rich Fibrin (PRF), the second generation of platelet concentrate was first developed by Choukroun et al in France. [7] PRF can be used in conjunction with bone grafts or as a membrane. [8] Several studies suggest that the combination of bone grafts and growth factors contained in PRF promote wound healing, bone formation and maturation, graft stabilization, wound sealing, hemostasis and improving the handling properties of graft materials. [9]

This novel biomaterial has several advantages over traditionally prepared platelet-preparation, represented as elimination of the risk associated with the use of bovine thrombin. In addition to decrease the time consumed in platelets concentrate preparation due to use easy technique. [7]

In order to evaluate the efficacy of new graft material, histological evaluation is valuable and more representative than radiographic evaluation but ethically cannot be obtained in human studies thus animal models can be used for this purpose. One of the experimental models which have been used for investigation of the effectiveness of different bone substitute materials is New Zealand white rabbits. This animal model is recommended for musculoskeletal research because they have less health problems and can withstand surgical trauma thus New Zealand white rabbits are the commonest used animal model for bone healing research. [10. 11]

II. SUBJECTS AND METHODS

A. Grouping

The present study included four rabbits with 16 defects. According to the graft used to fill the surgical defects, two study groups were carried out. Group (A) was grafted with BCP covered with PRF and consisted of 6 defects, while Group (B) was grafted with BCP only and consisted of 6 defects. Both groups have been compared with the control group which was assigned as Group (C) represented with 4 defects which was left empty.

B. Preoperative preparation

The experimental study was performed at animal house, Cairo University. Four white New Zealand male rabbits weighting 2-2.5 Kg. The animals were fasted for 7 hours preoperatively. Both tibias were shaved using a blade razor out the surgical room and the surgical field was draped using sterile drapes and the surgical site was cleaned using 10% povidone iodine (Povidone iodine 10% The Nile Co. for pharmaceuticals and chemical industries- Cairo A.R.E) and 70% alcohol swabs before manipulation. All the surgical procedures were performed under general anesthesia and the dose was calculated according to body weight. Xylazine (Robmpun, Bayer. Leverkusen, Germany) was administered as premedication (0.6 ml inter muscular) to keep the animal sedated followed by slow administration of Ketamine (Ketam 50, Hikma, Pharmaceutical Co.) 50-60 mg Kg intravenous (through the lateral auricular vein). Terramycin eye ointment was administered to lubricate the eyes. Mepivacaine with adrenaline (Mepivacaine -L Alexandria pharmaceuticals-A.R.E) was administered locally along the incision line. According to Bai, Meng-Yi et al [12], preparation of PRF membrane was carried out by withdrawal of five ml of blood from ear vein as shown in and centrifuged to prepare PRF membrane using centrifuge device (80-1, Desktop, Low speed, Electronic Centrifuge, China) Fig 1 (A, B).

C. Surgical procedures

Skin was incised using no. 22 blade then the fascia was cut with scissors until the bone is reached, then the periosteum was incised and then reflected using mucoperiosteal elevator. Two mono-cortical bone defects (diameter 5 mm, depth 5 mm) were created in each tibia using round surgical bur mounted on micromotor with copious irrigation of sterile saline. Each rabbit had four defects one defect left empty to represent the control group and three defects filled with either BCP (Guidor Easy-graft CRYSTAL products, Sunstar) only or BCP covered with PRF as intervention groups. Where the total defects were 16 defects in four rabbits divided into six defects grafted with BCP+PRF, six defects grafted with BCP only and four defects left empty as control

group Figure 1(C,D). The fascia was sutured using vicryl 4/0 (Assucryl, Assut sutures, Switzerland) while the skin was sutured using black silk 3/0.

D. Postoperative care:

A topical antibiotic Bivatricin Aerosol powder spray 150 ml, Egyptian company for advanced pharmaceutical was applied on the sutured incision line immediately postoperative and 3 times per day for 3 days postoperatively. An immediate postoperative one dose of Bupivacaine was administered around the wound. Nonsteroidal anti-inflammatory Ibuprofen (Brufen, Abott laboratories, Egypt) twice daily oral administration using a dropper for 5 days postoperatively. Enrofloxacin antibiotic was given orally using a dropper for 5 to 7 days. Animals were evaluated during 24 postoperative hours, then twice daily to check signs of pain, inflammation, wound dehiscence, and fractured leg.

Tissue preparation: The animals were decapitated after one month. Both femur bones were dissected and prepared for histological examination. The excised tissues were fixed in formalin 10%, decalcified in EDTA for four weeks and then processed and embedded in paraffin. Two slides of 4µm tissue sections were prepared from each specimen to be stained with hematoxylin and eosin (H&E) and Masson trichrome (MTC).

Microscopic examination: The slides were labeled A, B and C to be examined blindly under an ordinary light microscope by the pathologist. The MTC-stained sections were examined to detect mineralized bone tissue (stained red) and collagen and unmineralized osteoid tissue (stained green).

III. RESULTS

Microscopic examination of H&E-stained sections: In the studied groups, there was evidence of healing but with different degrees. The bone defects were closed by dense connective tissue with areas of bone trabeculae. The wound was filled by granulation tissue infiltrated by chronic inflammatory cells. Bone trabeculae with osteoblastic rimming and reversal lines were detected in the center of the lesion and at the periphery (Fig. 2a-c). Bone trabeculae were thicker in group A grafted with (BCP+BRF). In addition, cartilage reconstruction was also seen in same group (Fig. 2a).

In the control group, bone defect was filled with granulation tissue which was formed of numerous newly formed blood vessels, fibroblasts, collagen fibers and heavy inflammatory cell infiltrate (Fig. 2d). Microscopic examination of MTC-stained sections:

In group (A), the bone trabeculae filling the defect were thick and mostly mineralized (stained red) in addition to cartilage tissue formation. Areas of mature cartilage, characterized by less and widely separated chondrocytes, were detected (stained red). A newly formed unmineralized bone trabeculae (stained green) was seen covering the defect (Fig. 3).

In group (B), thinner bone trabeculae were observed in this group with less mineralization. The defect was covered by dense collagen fibrous band (stained green) (Fig. 4).

MTC was not applied to the tissue sections of the control group as no bone could be detected in microscopic examination of H&E-stained sections.

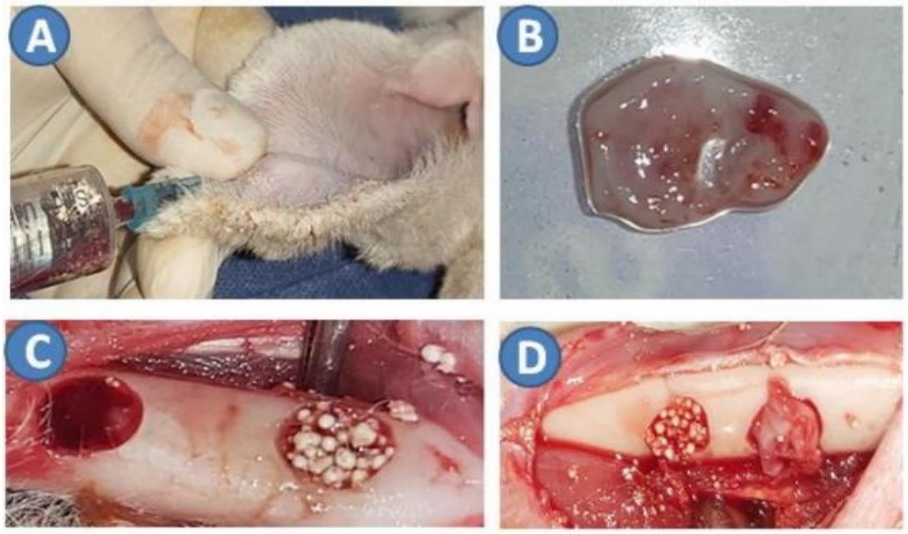

Figure (1): Preparation of PRF membrane (A), Five ml of blood was drawn from ear vein (B), PRF membrane, (C) Two monocortical bone defects one defect grafted with BCP the other defect left empty, (D) one defect was grafted with BCP covered with PRF membrane and other defect grafted with BCP only.

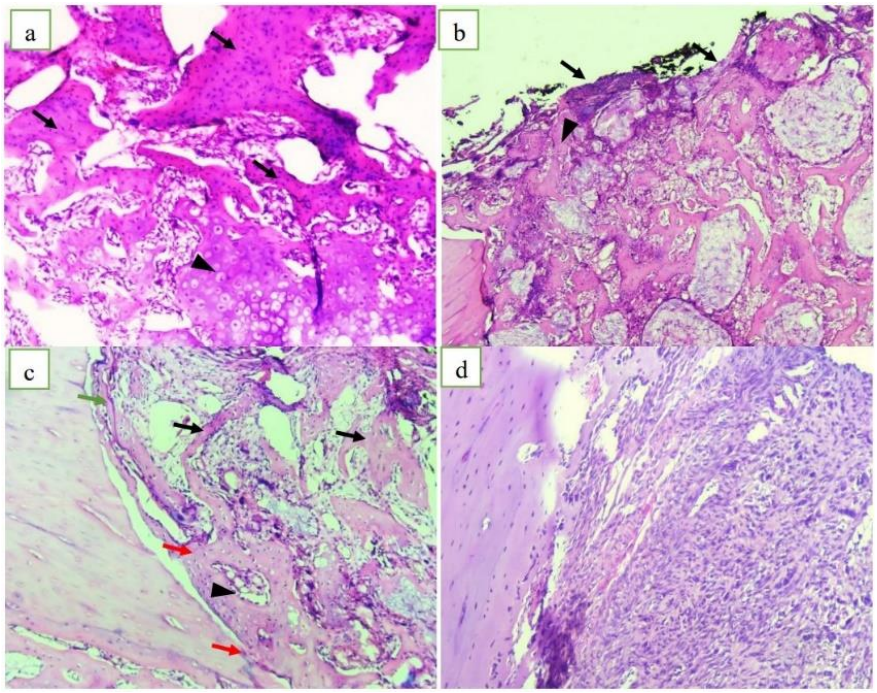

Figure (2): Photomicrographs of the intervention groups showing the healing of bone defect with granulation tissue and bone trabeculae. (a) bone defect of group A filled with thick bone trabeculae (black arrows) and cartilaginous tissue (arrowhead) were seen (H&E x100). (b) bone defect of group B covered by dense connective tissue (arrows) and thin bone trabeculae extending just beneath the surface (arrowhead) (H&E x40). (c) bone defect of group B showing the fusion of newly formed bone with marginal bone (red arrows), osteoblastic rimming (arrow head) and reversal lines (green arrows) (H&E x100). Note bone trabeculae are thicker in group A than B (black arrows) in (a) and (c). (d) bone defect of control groups filled with granulation tissue, heavy inflammatory cell infiltrate and no bone formation (H&E x100).

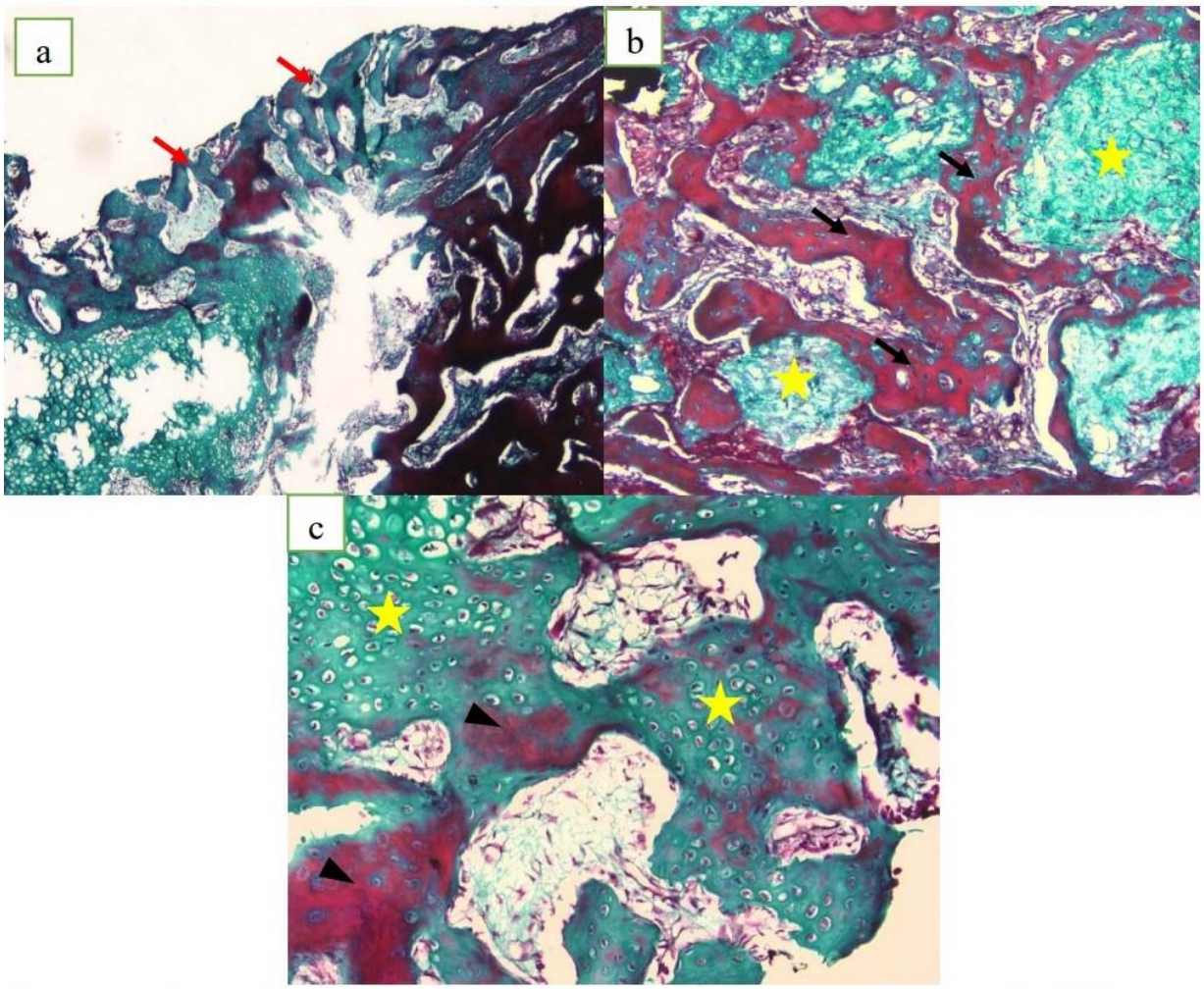

Figure (3): Photomicrographs of group A showing (a) a continuous shelf-like layer of bone trabeculae covering the bone defect (red arrows) (MTC x40), (b) a granulation tissue filling the bone defect (stars) with thick and mineralized bone trabeculae (black arrows) (MTC x100), Note cartilage formation (stars) with areas of maturation (arrows) (MTC x100).

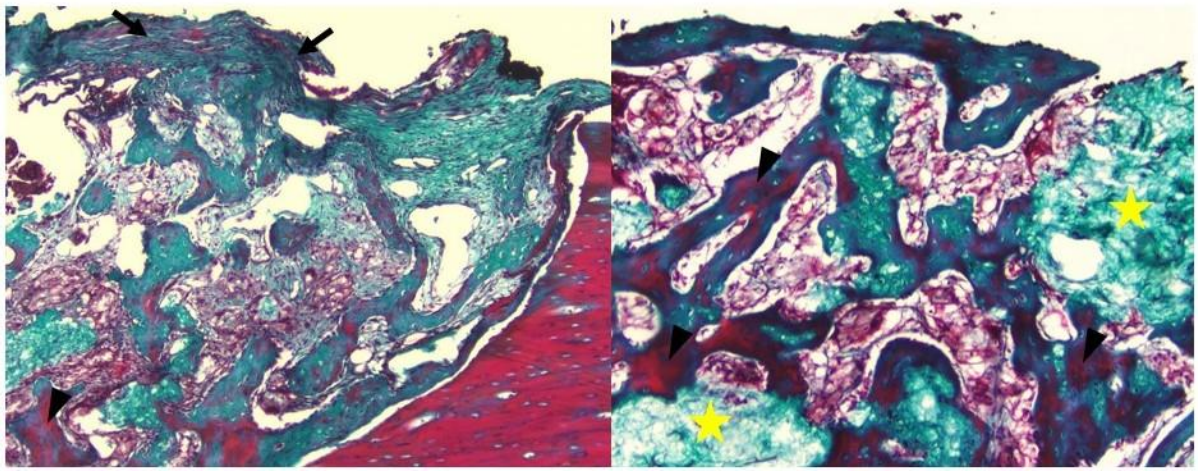

Figure (4): Photomicrographs of group B showing dense fibrous band covering the defect (black arrows). A newly formed unmineralized bone filling the defect margin and extending beneath the collagen fiber band. Note small areas of mineralization (arrow heads) (MTC X40). A higher magnification showing granulation tissue filling the bone defect (stars). Partially mineralized bone trabeculae (arrow heads) were observed (MTC x100).

Table (1): Summary of MTC results.

	Group A	Group B
Defect surface	Shelf-like bone trabeculae	Dense band of collagen fibers
Bone trabeculae inside the defect	Thicker and mostly mineralized	Thinner and less mineralized
Cartilage formation	Observed	Absent

IV. DISCUSSION

Although autogenous bone graft has been used extensively to reconstruct bone defects but with less than ideal results, due to its drawbacks, Such as significant resorption, limited amount of bone and donor site morbidity. Many studies have been conducted to evaluate various bone substitutes in order to avoid these adverse effects, however many controversies still exist to decide that whether artificial bone substitutes can be considered an alternative to autogenous graft or not. (3)

Biphasic calcium phosphate (BCP), mixture of hydroxyapatite (HA) and B-Tricalcium phosphate (B-TCP) is a synthetic bone substitute which has the native composition and mechanical features of natural bone. (13) This biocompatible material which is widely used in orthopedic, maxillofacial, and periodontal surgery, has mainly osteoconductive properties. (4, 5, 14) This material could be combined with other factors that has osteoinductive properties. Recently platelets preparations have been used to enhance osteoinductive properties due to their contents of multiple growth factors.

The presented study evaluated bone formation and regenerative potential in surgically created bone defect filled with BCP with and without PRF. Male white New Zealand rabbits were chosen because of the ease of handling, less aggressive in nature, less health problems compared with other breeds. (15) In the present study monocortical defects was drilled in tibia with a diameter 5 mm according to most other authors used monocortical drill holes with a diameter of 4.5–8 mm and a length of 6.5–10 mm, (16-19) as even monocortical defects with a diameter of 4 mm were reported to be critical sized. This size was selected that permit doing multiple bony defects without endangering strength of the bone.

Calcium phosphate ceramics have been widely applied as bone substitutes due to their resemblance to the mineral portion of the bone tissue, biocompatibility, unlimited availability and bioactivity with native bone. All these properties make them a good choice for being an alternative to autogenous bone. The main limitation of this promising material is lacking of osteoinductivity. Many previous studies used composite graft consisted of calcium phosphate combined platelet concentrates to enhance bone regeneration. In the present study BCP as one of calcium phosphate ceramics and the second-generation platelet concentrate PRF were evaluated histologically to evaluate its efficacy as alternative bone substitutes.

The results showed that BCP combined with PRF showed thicker mineralized bone trabeculae than BCP group. These results are consistent with the results obtained from animal study performed by. (20) In which implantation in bony and ectopic sites revealed that a composite of BCP and blood clot had osteogenic properties. It was able to repair a 6-mm critical femoral defect in rat and induced woven bone formation after subcutaneous implantation. In the same line, (21) a histologic and histomorphometric study evaluated the efficacy of PRF combined BCP in grafting surgically created bone defects on six sheep's tibia. Bone defects were divided into 4 equal groups. Three test groups grafted with BCP, PRF, BCP + PRF respectively and one control group left empty. They were evaluated during 10, 20 and 40 days follow up periods. The results revealed increase in bone formation with addition of PRF to BCP more than the other groups. At day 40, newly formed bone occupied 50% of bone defect and the trabecular structure of newly formed bone was observed around BCP granules. This composite graft gave promising results. However, it needs further investigations on a larger defect and a longer follow up period.

On the other hand, cartilage formation was one of the important finding in the bone defect treated with BCP combined with PRF, a result that provides an excellent biomaterials for heterogenous tissue degeneration. In the past few years, PRF has introduced as a promising non-invasive agent in the treatment of cartilage injuries. It was found that PRF

improved the healing of knee cartilage defects in different animal models. However, its benefit in cartilage repair is not fully understood. (22)

V. CONCLUSION:

Biphasic calcium phosphate combined platelet-rich fibrin provides an excellent graft material for faster bone regeneration, compared to Biphasic calcium phosphate alone. Further study is recommended to evaluate its effect in inducing cartilage repair.

Conflict of Interest:

The authors declare no conflict of interest.

Funding:

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors

Ethics:

The study was approved by the Animal Ethical Committee of Cairo Univeristy (no.CU III F 7 23). All the surgical procedures were performed according to the Cairo University ethical guidelines for welfare of experimental animals. The study was conducted at animal house, El Kasr el Aini. The study included 4 rabbits (3-3.5 kg weight) the surgical procedures were performed under general anesthesia under sterile conditions.

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