Comparison between the Use of Platelet Rich Fibrin with/and Without Biphasic Calcium Phosphate for Osseointegration around Implants (Experimental Study)

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Abstract: ***Background: Dental Implants have changed the face of dentistry over the last 25 years. The clinical replacement of lost natural teeth by osseointegrated implants has represented one of the most significant advances in dentistry. They developed a variable alternative to conventional prosthetic reconstruction of edentulous areas. The goal of modern dentistry is to restore natural contour, function, comfort, aesthetics, speech, and health. Advanced researches in implants designs, materials and techniques have led to predictable success in their application. Study objective: This study will evaluate the effect of osseointegration and bone formation of biphasic calcium phosphate with platelet rich fibrin (PRF) around dental implants in experimental dogs. Methods: Eight mongrel dogs will receive dental implants, in which (group B) four dogs will receive dental implant on the left side with Platelet Rich Fibrin and Biphasic calcium phosphate (study group) and (group A) four dogs will receive dental implants on the right side with Platelet Rich Fibrin alone (control group). Two dogs will be sacrificed at 2, 4, 6 and 8 weeks one from each group and speciemens will be dissected to be histologically evaluated for new bone formation around implants and osseointegration. Results: the healing outcome here appeared consistent and bone formation was greater filling almost the whole of the created cavity in group B and the greater part of that cavity in group A with the same superior blood supply in group B than that in group A reflecting great neoangiogenesis. In both groups the formed mass of the new bone exhibited striking appearance of osteointegration with the native bone. The better positive results obtained in association with group B in all of the experimental periods suggest a superior effect of PRF when used in association with BCP as a bone graft than if PRF used alone. Conclusion: Within the limitations of this experimental study, it can be concluded that PRF in addition to BCP may favor the formation of new bone. The effectiveness of PRF depends not only on its features but also the properties of coadministered grafting material.***

Keywords: PRF, Biphasic Calcium Phosphate, Osseointegration, Implant, Centrifuge

1. Introduction

The dental professional must use considerable clinical skills to help patients cope with the effects of partial or complete edentulism. Dental problems that were historically the most difficult can be solved today with the assistance of dental implants [1].

When an implant is placed immediately into an extraction socket it may engage the walls of the socket near the crest of the alveolar ridge with presence of bone defects around the implant. Ingrowth of soft tissue could compromise the achievement of osseointegration in the crestal bone area [2].

Various bone graft materials have been developed and used for reconstruction of bony defects in periodontal and implant surgeries. The autogenous bone is considered as the gold standard because of its superior capacity in bone formation, however, has some clinical drawbacks such as a morbidity of donor site and uncontrolled resorption rate [3,4].

Indeed, they have shown favorable biocompatibility and osteoconductivity when used as bone graft materials [5].

Among Ca-Ps, HA, which is very stable, can maintain the space effectively but has low osteoconductivity [6,7].

In contrast, β-TCP is more biodegradable and rapidly replaced by newly formed bone but has low capacity of space maintaining [8].

Therefore biphasic calcium phosphate (BCP), which is composed of HA and β-TCP, was introduced to overcome limitations of each material and several studies have been demonstrated that BCPs can be used as bone substitutes successfully [8-10].

Calcium phosphate materials have received a lot of research attention in recent years due to their chemical similarity to bones and teeth. They are attractive biomedical materials owing to their excellent biocompatibility and the nontoxicity of their chemical components [11-14].

Calcium phosphates belong to the group of bioactive synthetic materials and its most frequently used are the hydroxyapatite and the tricalcium phosphate. These types are commonly used due to their osteoconductivity, crystallographic structures, and chemical composition similar to the skeletal tissue. They are classified according to their "resorbability," that is extent of degradation in vivo. Hydroxyapatite has been described as "nonresorbable" and tricalcium phosphate has been described as "resorbable" [13,14].

Platelet-derived growth factors are biologically active peptides that enhance tissue repair mechanisms [15]. Platelets are easily collected from the blood stream and are concentrated in a small volume of plasma known as platelet-rich plasma (PRP) [16].The preparation of PRP involves the isolation of PRP after which gel formation is accelerated using calcium chloride and bovine thrombin.

Platelet rich fibrin (PRF) is a second generation platelet concentrate widely used to accelerate soft and hard tissue healing. The advantage of platelet rich fibrin over the better known platelet rich plasma include ease of preparation, application, minimal expence and lack of biochemical modification [17-19].

A combination of PRF and biphasic calcium phosphate (HA/ β-TCP) is expected to create a composite with both osteoconductive and osteoinductive properties

2. Materials and Methods

Pre-operative phase
The animals were examined by the animal house veterinarian to exclude any diseased animal.
All dogs were kept under the same environmental condition.
All dogs received the same balanced diet through the period of the study (milk, meat, broth).
All animals received a dose of antibiotic intramuscularly: ampicillin 25/kg just before the operation.

Operative Phase
The animals were generally anesthetized via intravenous injection of Sodium Thiopentone; the dose of which was calculated on basis of 30mg/kg body weight.

The oral mucosa was painted and rubbed thoroughly with antiseptic Povidone Iodine solution 10% so as to render the surgical field free from microorganisms.

Collecting 10ml of blood from the dog vein, The required quantity of blood is with drawn into a 10/ml test tube without anticoagulant blood is centrifuged using tabletop centrifuge for 12 min at 2,700 rpm.

Removing the top and bottom layers to get only PRF layer in the middle of the prepared sample, then squeeze it between two glass slaps to get a membrane of PFR to be used. (Fig. 1)

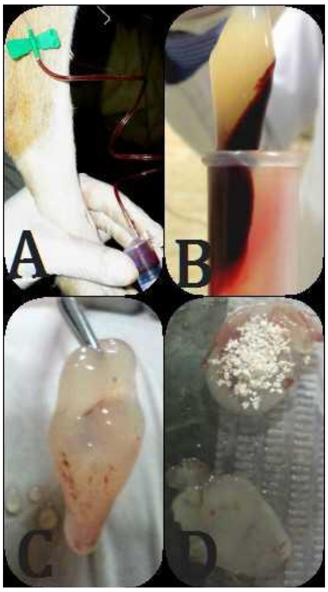

Figure 1: A: Showing blood sample, B: blood after using centrifuge, C: PRF only after removing top and bottom layers, D: two halfs of PRF one containing BCP

The lower right premolar tooth was extracted with minimal trauma using a lower premolar forceps. (Fig. 2 A)
Drilling with the oeteotomy drill mounted on low speed handpiece was done in the socket under cooling with sterile saline irrigant. A pumping motion wasused while drilling to allow the osseous debris to be cleared from the site. (Fig. 2 B)

Preparing the socket using half of the PRF inserted into the socket before inserting the dental implant. (Fig. 2 C)

The implant was inserted into the prepared socket with the vial cap and turned in a clockwise direction with slight apical pressure to gain stability of the implant in its position till difficulty is encountered, and then the vial cap was removed. (Fig. 2 D)

For final seating of the implant in bone; the Ratchet driver combined with the hex driver was mounted onto the implant, and used till the implant body was flushed with the level of alveolar crest of bone.

Wrenching was carried out in only a horizontal plane, and care was taken to avoid movements in any other planes, not to widen the osteotomy.

The cover screw was then placed on top of the implant using the finger driver.

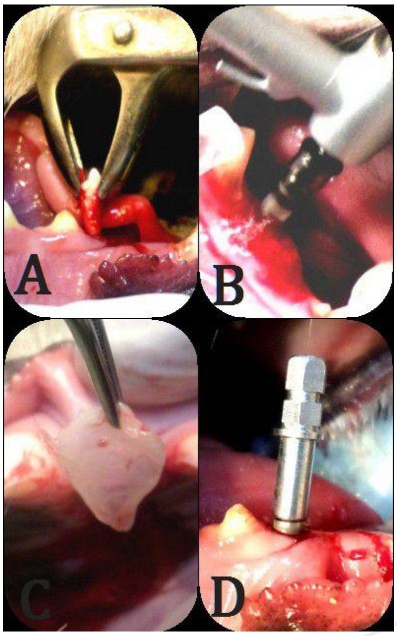

Figure 2: A: premolar extraction, B: socket drilling, C: preparing socket with PRF, D: implant insertion

An interrupted suture was done to approximate the gingival margins using 3-0 resorbable suture material.

The same procedure was carried on the left side except adding BCP (DM bone) with the half of the PRF membrane. (Fig. 3)

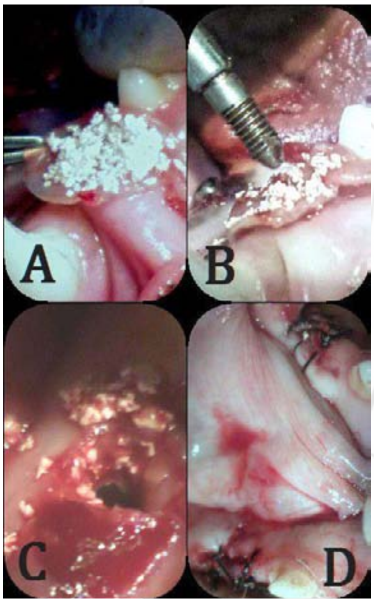

Figure 3: A: preparing socket with PRF + BCP, B: implant insertion, C: after implant fixation, D: wound closure in both sides

Animal Sacrification

Two Dogs were sacrified at 2, 4, 6 and 8 weeks postoperatively by giving them an overdose of thiopentone sodium intravenously. Segments containing the grafted areas and the adjacent bone were retrieved to be prepared for histological examination. They were immediately immersed in 10% neutral buffered formalin for fixation.

3. Results

Clinical Result

All animals survived well, and remained active and alert all over the course of the experiment. During the first week the animals did not exhibit any clinical signs of infection. In the second post-operative week; the clinical observation revealed that the wound showed no difference in healing features for both groups.

Six weeks post operatively; there were no sign of graft rejection, also no depressions were noted in the level of bone facially on either side.

Eight weeks post operatively; the continuity of the bone of the body of the mandible at the surgical sites was the same in both sides.

Histological Results

First observation period (after two weeks from the start of the experiment)

In both groups, in some sections inflammatory reactions were observed, with some areas of resorption of the boundary of the native bone. Other sections exhibited features of new bone formation and new vascularization which were more in association with group B (PRF+BCP) than in group A (PRF).

Second observation period (4 weeks after the start of the experiment)

Resolution of inflammation in the healing tissues was noted in both groups together with formation of more amounts of new bone and vascularization than in the first observation period. They were both more pronounced in group B than in group A. The blood supply to the healing mass was very outstanding in group A which also exhibited better figures of osteoblastic activity and osteocyte incorporation in the forming bone. Also, in group B the newly formed bone appeared more organized than in group A.

Third observation period (6 weeks after the start of the experiment)

Large number of osteocytes within the newly-formed trabeculae. Evident remodeling lines were seen between the native bone at the implant site and the new bone and it is more dominante in group B than group A

Fourth observation period (8 weeks after the start of the experiment)

In contrary to the variations of the histological features in the previous two experimental periods, the healing outcome here appeared consistent and bone formation was greater filling almost the whole of the created cavity in group B (Fig. 4 A&B) and the greater part of that cavity in group A (Fig. 4 C&D) with the same superior blood supply in group B than that in group A reflecting great neoangiogenesis. In both groups the formed mass of the new bone exhibited striking appearance of osteointegration with the native bone.

The better positive results obtained in association with group B in all of the experimental periods suggest a superior effect of PRF when used in association with BCP as a bone graft than if PRF used alone.

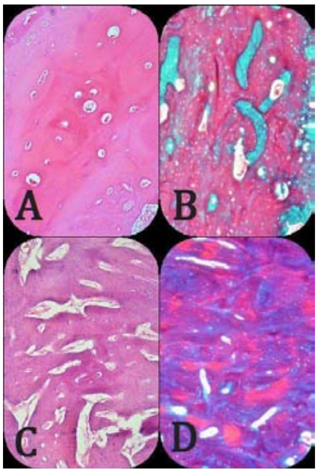

Figure 4: A: H&E stain L.M of study group, 8 weeks, B: trichrome stain L.M of study group, 8 weeks, C: H&E L.M of control group, 8 weeks, D: trichrome stain L.M of control group, 8 weeks.

4. Discussion

The present study was designed to evaluate histologically the effect of platelet rich fibrin (PRF) combined with biphasic calcium phosphate (BCP) as bone filler in bony defects of dog mandibles. Since the literature includes few histological studies using only PRF or graft materials with different characteristics combined with PRF.

Various platelet derived products or platelet concentrates have been introduced that act as biological mediators aiding the healing response. Choukroun's PRF has been the latest development among the platelet concentrates. It is a second-generation platelet concentrate widely used to accelerate soft and hard tissue healing and is a strictly autologous fibrin matrix containing a large quantity of platelet and leukocyte cytokines. Thus PRF has also been tagged as a healing biomaterial [20].

Alloplastic grafting products such as biphasic calcium phosphate (BCP) are synthetic in origin, biocompatible and osteoconductive [21]. The release of controlled levels of calcium ions over time favors the formation of an apatite layer, which is necessary for the bioactivity displayed in BCP ceramics [22,23]. This bioactivity can be responsible for the ceramic's osteoconductivity and/or osteoinductivity. In osteoconduction, the biomaterial surface supports the growth of mature osteoblasts and direct apposition of bone into its surface while in osteoinduction, the biomaterial favors the recruitment of immature or undifferentiated cells and stimulates their differentiation towards the osteoblastic lineage and as a consequence, osteogenesis will be stimulated. Therefore clinically applied BCP with PRF was preferred in this study.

The present study was conducted on eight healthy dogs. In each dog tooth extraction were performed; one on the right and the other on the left. In group A (right side) the animals received PRF only and in group B (left side) the animals received PRF mixed with BCP. These dogs were sacrificed at 2,4, 6 and 8 weeks postoperatively for histological evaluation of the healing process.

In the present study, the observations of the first experimental periods in both groups revealed that an inflammatory reaction occurred. Although the inflammatory cells were denser in group A, the inflammation was not prevailing throughout the depth of the healing defects. It seemed that the regenerative potential of the tissue biology could limit and control the spread and continuation of inflammation.

Also, Zhang et al (2012) [24] conducted histological and clinical evaluations of 10 patients who underwent sinus lifting. As a test group, six sinus floor elevations were grafted with a Bio-Oss and PRF mixture, and as control group, five sinuses were treated with Bio-Oss alone. Their results revealed that there was no difference in the new bone between the group receiving only bovine bone graft (Bio-Oss) and that receiving PRF in combination with bovine bone graft 6 months after sinus-lifting surgery.

Contrary to the results of these studies, we observed higher new bone in group B (PRF+BCP) than group A (PRF alone) during the four observation periods. The positive reaction in group B was preceding that of group A. This proves that PRF is effective in the early stages of healing.

In 2013, Bölükbaşiet al [21] evaluated the efficacy of PRF mixed with BCP on bone regeneration in surgically created bone defects in sheep tibia.The defects were left empty or grafted with BCP, PRF, or BCP+PRF. Animals were killed at 10, 20, and 40 days. The specimens were analysed histologically and histomorphometrically. The study revealed a histomorphometric increase in bone formation with the addition of PRF to BCP.

In the present investigation, during the four observation periods, the healing features in group B were different from those in group A in two obvious points; the blood supply and the amount of the newly formed bone, both were greater in group B than in group A especially the profound blood supply.

The combination of PRF with autogenous bone or bone substitute materials are opening the door to novel therapeutic alternatives and improving preexisting ones, increasing the versability of bone substitute materials. PRF acts as a biologic adhesive to hold the particles of the graft material together, making its manipulation much easier, providing vascularization and survival of the graft [25].

The effectiveness of PRF depends not only on its features but also the properties of coadministered grafting material [21]. The better positive results obtained in association with group A in all of the experimental periods suggest a superior effect of PRF and rapid start of bone formation when used in association with BCP as a bone graft than if used alone.

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