The Clinical, Histological and Histomorphometrical Evaluation of Decalcified Freeze-Dries Bone Allogenic Graft (DFDBA) with Plasma Rich Growth Factor (PRGF) for Alveolar Ridge Preservation

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ABSTRACT

Background: The aim of this study is to compare the clinical, histologic and histomorphometric evaluation of decalcified freeze dries bone allograft with and without plasma rich growth factor on extraction socket preservation. Methods: This randomized, controlled clinical trial was carried on 10 cases with a non-restorable tooth requiring extraction and planned to place dental implant. Subjects were divided into two groups: test group received DFDBA in conjunction with PRGF and collagen membrane, control group received DFDBA alone with collagen membrane. Vertical socket dimensions were measured immediately after extraction and three months later. Prior to implant placement, bone core samples were retrieved from the center of the healed socket for histologic (amount of vascularization and inflammatory infiltration and histomorphometric (new area of bone formation, trabecular thickening, remanent biomaterial) analysis. Data were statistically analyzed using T tests and Mann-whitney U test. Results: According to the results, area of bone formation was 62.5% ± 3.2% in test group compared to 31.7% ± 1.1% in control group (P=0.000), trabecular thickening more in test group incompared to control group (48.08 ± 10.37, 28.22 ± 2.83 H respectively) (P=0.003) and remanent biomaterial in test group compared to control group were respectively 0.03%±0.020, 0.08%±0.45(P=0.03). No significant difference were found in clinical and histological finding between groups. Conclusion: The histomorphometric findings suggest that PRGF enhanced bone regeneration more than PRGF-free graft. Although no clinical and histological difference were found.

INTRODUCTION

Nowadays the dental implant is the most frequent treatment modality to recover the function of the edentulous jaw [1]. The volume and density of the alveolus is crucial in implant site [2, 3]. Healing of extraction sockets is usually associated with the loss of residual ridge height and width. It is hard to place an implant in area with significant bone resorption. Post extraction preservation of the alveolar ridge allows to place an implant with suitable esthetic and functional criteria [4-8]. Alveolar ridge resorption following tooth extraction may result in 40% to 60% loss of bone height and width within 2 to 3 years. Boneresorption may result from anatomic, prosthetic, metabolic, and functional factors [9]. Misch et al speculated that the loss of alveolar ridge bone height and labial plate after tooth extraction is due in part to the constriction of the blood clot within the alveolus and the thin labial cortical plates remodeling in response to impaired blood supply after the extraction [10]. The use of bone substitute and GBR techniques have been shown to enhance socket healing and to reduce the resorption process. Alveolar ridge augmentation procedures are frequently used to increase ridge height and width prior to dental implant placement. Currently there are some case reports of ridge augmentation results. The results showed the positive treatment effects [11].

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Site preservation following tooth extraction through bone grafting helps to reduce the dimension changes thereby reducing resorption of vertical bone height and prevention of collapse soft tissues at the site. This technique is crucial to obtain optimal esthetic and functional restoration results. Generally implant placement in a healed socket has got a higher degree of predictability and stabilization soft tissue contour. Studies have shown the beneficial effects of the use of regenerative biomaterials in augmenting the alveolar following extractions including autogenous, allogenic, xenograft, and alloplast sources [12, 13]. Nowadays, alloplastic materials have a major role in bone repair due to the success in space maintenance, rapid bone turnover, biocompatibility, and no need to harvest from another site [12-19]. Demineralized bone matrix (DBM) is a biomaterial which is a production of Iranian Tissue Bank Research and Preparation Centre Imam Khomeini Medical Complex, Tehran, Iran. Preparation of demineralized bone matrix Human long bone shafts were acquired with aseptic techniques from cadaver, routine microbial and viral tests were accomplished according to FDA and AATB protocols. The cortical shafts and cancellous part were debrided, stripped off periosteum, and treated to remove lipid, blood, and cellular remnants before being frozen to −70 C and segmented. The bone segments were immersed in an ethanol and then ethyl ether to cover the tissue. They were rinsed with water and sent to mixing with ethanol. Then, it was mixed with HCL. Next, it was rinsed with PBS until reached pH 7-7.4. Finally, the resulting powder was lyophilized, packed and sent for irradiation with 25 Kgy gamma rays [20, 21].

In a study performed by Khoshzaban et al. in 2011, three demineralized bone-matrix allograft materials including ITB, β TCP, Bio OSS on inflammation and bone formation in defects made in mice calvaria are compared, and the highest bone formation occurred in ITB-DBM group [20]. The use of biologically active endogenous proteins for regenerative purposes, has performed a new way for tissue regeneration. In 1999, Anitua [22] described a new method to prepare platelet-rich plasma called PRGF. This is a 100% autologous procedure which is rich in biologic mediators to accelerate hard and soft tissue regeneration. Adhesive molecules derived from plasma, such as fibrinogen, fibronectin, vitronectin, and thrombospondin-1 act as matrix or scaffold and absorb platelets and stem cells. Platelets are rich source of growth factors including PDGF, TGF-B, VEGF, FGF, insulin like growth factor (IGF) and GM-CSF [22-24]. Plasma rich in growth factor (PRGF) is an autogenous plasma product which releases some growth factors and bioactive proteins following calcium activation, and accelerates wound healing and tissue regeneration process [22]. Plasma rich in growth factor is derived from the patient’s own blood using simple methods, and is promptly used in surgery location after preparation. Since PRGF contains high concentration of growth factor, can cause accelerate or increase regeneration [26]. In a research done by Behniaet al. (2012), histologic evaluation of the effect of DBM with Mesenchymal stem cells and PRGF on bone regeneration, the results showed that PRGF and MSCs had positive treatment effects [25].

In another research which done by Anitua et al [24], for evaluating the effect of PRGF in combination with allograft materials on osteo-regeneration, showed that plasma rich in growth factors improves the condition required for osteo-regeneration in implant installation site. So this study was aimed to compare DFDBA alone with DFDBA in conjunction with PRGF in socket preservation clinically, histologically and histomorphometrically.

**MATERIALS AND METHODS**

**Case selection:**

In this experimental, randomized, controlled clinical trial study, 10 subjects with a non-restorable tooth requiring extraction which candidates for the socket preservation and subsequent implant replacement in mandibular jaw were selected. After ethical approval, ten dental sockets were divided in two groups including test group and control group. DFDBA with PRGF and absorbable membrane collagen for test group, and merely DFDBA and absorbable membrane collagen for control group were used, and the effect of PRGF was evaluated. Patients were chosen that were being healthy; non smoker patients; had good cooperation; were available for follow up courses, and accepted their cooperation on this project in written. Patients with debilitating systemic diseases or diseases that have a clinically significant effect on the periodontium (ex. un controlled diabetic disease ,immune disease …); history of oral anticoagulant ,immune suppressor drug and intravenous bisphosphonate use or oral bisphosphonate use for >3 years; pregnancy; known allergy to any material used in the study; heavy smokers; previous head and neck radiation therapy; chemotherapy in the last 12 months; severe psychologic problems; poor oral hygiene; or had not good cooperation were excluded.

**Clinical and Radiographic Parameters:**

Prior the surgical procedure, each patient received a standardized periapical radiographs clinical photographs at baseline and after 3 months. Occlusal stents were fabricated on the study casts to serve as fixed reference guides for the vertical measurements. Vertical distance were measured mesial, distal, mid buccal, and lingual surfaces using a 15-mm periodontal probe immediately after extraction. The same guide was used at the implant placement time to measure the amount of vertical dimension changes at the same four sites from. All
clinical measurements were recorded by the same examiner. The difference in the measurements for all surgical sites at baseline and at 3 months, provided a clinical evaluation of the amount of bone resorption. Prior to implant placement bone core samples were retrieved from the center of the healed socket for histologic and histomorphometric.

**PRGF preparation:**

To PRGF preparation, at least 20-milliliter arterial blood was taken and the samples were preserved in sterile blood collecting tubes containing half a milliliter anticoagulant agent (sodium citrate 3.8%). Based on technical standards (PRGF), blood sample was initially centrifuged for eight minutes at 460g velocity. The blood segregates into four parts, the higher part of the tube (1ml) is plasma poor in growth factors (PPGF), then (0.5 ml) plasma growth factors (PGF), Plasma rich in growth factors (PRGF) immediately (0.05 ml) above white blood cells (WBC) and red blood cells (RBC). For this study, PRGF was used. PRGF was taken by half a milliliter pipettes and transferred to a separate tube and was used for soaking allograft material.

**Surgical Procedure for Test Group:**

Patients received prophylactic antibiotics and 0.12% chlorhexidine rinses 30 minutes prior to surgical procedures. Following administration of local anesthesia, the extraction was carried out atraumatically (with flapless technique and separation of periodontal ligaments around the teeth and carefully luxation using periostome). The socket was debrided to remove all remnants of the periodontal ligament and inflamed tissues. Socket walls were measured by using guide at four sites and recorded. DFDBA with PRGF was mixed and lightly packed into the extraction socket with a flat plastic instrument. The site was covered with resorbable collagen membrane to prevent the collapse of the surrounding soft tissue into the socket during the healing process and was secured using a reverse cross-mattress resorbable suture. Post-operative care comprised of 0.12% chlorhexidine rinses twice daily for 4 weeks, systemic antibiotic (amoxicillin 500 mg every 8 hourly) for 1 week, analgesic medication (Ibuprofen 400 mg every 8 hours) for 3 days. After 3 months clinical evaluation was performed and a Panoramic and CBCT radiograph from the site was taken. Following local anesthesia full-thickness flap was reflected. Clinical measurement was made using the same guide used previously at baseline at the same four sites to recorded bone-wall height. A small core of newly formatted bone was removed from the center areas of the sockets using a trephine (3-mm-outside-diameter,2-mm-inside-diameter) with irrigation at a bur speed of 1,000 rpm. The samples had been collected and placed immediately in 10% formic acid so the samples had been collected and placed immediately in 10% buffered formalin. A dental implant was placed in the socket subsequent to performing a complete osteotomy according to the recommendations of the manufacturer. Healing abutments or cover screws were placed based on primary stability of the implant, and flaps were secured with 4-0 PGA sutures.

Surgical protocol for the control group patients was followed exactly as for the test group except that the extraction socket was received DFDBA alone with resorbable collagen membrane (Fig. 1).

**Histologic and Histomorphometric evaluation:**

The biopsies taken from human jaws were transferred to 10% formalin solution and kept for at least ten days for complete fixation. Then those were transferred to 10% formic acid solution and kept for one week in that solution. In this period they were inspected in a daily basis to control decalcification. After that, samples were taken off the formic acid solution and were soaked for five minutes in 20% lithium bicarbonate solution to neutralize acid. Each sample was marked with a number. Finally, bone samples were cut vertically two halves. Central part of the bone was marked by Indian INK. The samples were placed in paraffin blocks. Five micron slides were gotten from paraffined blokes and were stained by hematoxylin-eosin method. Then the microscopic slides were evaluated histopathologically and histomorphometrically by an oral pathologist using Olympus BX41 light microscopic. In histopathologic study grade of inflammation, bone trabecular thickness, type of bone-biomaterial connection (existence or absence of connective tissue between bone parts), area of bone formation and number of blood vessels were evaluated. It is worth to mention that the numbers of blood vessels are evaluated in three scopes at 40x zoom level, and scored as [26]:

Less than three blood vessels considered as ‘zero’, between three and five blood vessels were ‘one’ and more than five blood vessels considered as ‘two’.

Grade of inflammation in five grades was evaluated [27]:

- Grade 0: Absence of inflammatory cells
- Grade 1: Little and scattered inflammatory cells (slight)
- Grade 2: Five to ten inflammatory cells (focal)
- Grade 3: Eleven to fifty inflammatory cells (Focal)
- Grade 4: More than fifty inflammatory cells (severe inflammation)

For histomorphometric Evaluation, photographs were taken from all sections taken from grafted area by camera, Olympus DP12, Tokyo, Japan and attached to light Olympus microscope at 40x magnification. The
images were assessed by SIS LS Starter software in jpeg format. Then area of bone formation were measured and percentage of bone formation to total area of the image were calculated. Also bone trabecular thickness was measured, including three grades 27:
Grade I: More than 60 microns (Thick)
Grade II: Between 21 to 60 microns (medium)
Grade III: Between 1 to 20 microns (Thin)
To prevent any bias in histologic and histomorphometric interpretation, in none of the steps the pathologist was aware of biopsy content. To precise assessment of measures in this method, seven sections were selected from each biopsy, and their mean value eventually reported as definite result.

RESULTS AND DISCUSSION

Data Analysis:
In this study, data were analyzed using SPSS software, version 18.8. Clinical values in first and second surgical steps were statistically evaluated using Mann-Whitney U test and T test. P<0.05 was considered significant.

Results:
Clinical Findings:
There was no statistically significant difference in vertical bone resorption for either study groups. Three months later, the extraction site was healed without any adverse event and free of infection or symptoms in both groups; also complete soft-tissue closure was present 14 days after extraction. Membrane exposure did not compromise the early stage of soft-tissue healing.

Fig. 1: The average vertical bone loss in test and control group are summarized.

Histomorphometric analysis:
The results indicated that there was a statistically significant difference between the two groups based on: area vital bone regeneration which was 62.5% ± 3.2% in test group compared to 31.7% ± 1.1% control group (P=0.000), trabecular thickening more in test group in compare with control group(48.08 ± 10.37, 28.22 ± 2.83 μ respectively) (P=0.003) and residual biomaterial in test group compared to control group were respectively(0.03% , 0.08% ) (P=0.03). Histomorphometric analysis is summarized in Table1.

Table1. Histomorphometric Analysis.

<table>
<thead>
<tr>
<th></th>
<th>Test group mean±SD</th>
<th>Control group mean±SD</th>
<th>P-value</th>
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<td>Trabecular thickening</td>
<td>48.08 ± 10.37</td>
<td>28.22 ± 2.83</td>
<td>0.003</td>
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<td>Area of bone formation%</td>
<td>0.62±0.03</td>
<td>0.31%±0.011</td>
<td>0.000</td>
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<tr>
<td>Residual biomaterial</td>
<td>0.03%±0.020</td>
<td>0.08%±0.45</td>
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Histologic findings:

<table>
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<th>Control group</th>
<th>blood vessels score</th>
<th>bone-biomaterial contact *</th>
<th>Vitality**</th>
<th>foreign body reaction***</th>
<th>inflammatory infiltration grade</th>
<th>Number of case</th>
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<td>5</td>
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</table>
In this study DFDBA allograft was used as a useful substitute for autogenous bone. The results of this study showed that the clinical mean value of vertical ridge analysis in four distinct regions of dental socket in test group was lower than control group, but the difference is not statistically significant. Histomorphometrical mean value of trabecular and area of bone formation percentage was higher in test group than control group, and residual biomaterial was lower in test group compared with control group and showed a statistically significant difference. Histologically, mean value of blood vessels was higher in test group than control group. In 20% of slides, the number of blood vessels was grade 1 and in 80% was grade 2. According to these results, no foreign body reaction was found in samples, inflammation was slight, and the bones were vital in all samples. A direct contact between biomaterial and bone was observed in all samples. In histomorphometric studies on microscopic sections, aside from numerous benefits, having a two dimensional image of a three dimensional space puts limitations on studying and analyzing of histologic sections of osteogenesis process [29]. Therefore, it can be mentioned that beside the effects of biologic factors on trabecular bone, some technical problems such as producing sections in longitudinal direction of the defect (perpendicular or parallel) are definitely affecting the resulted microscopic image and this can explain the existing difference in study results. On the other hand, reported histomorphometric values should be compared and analyzed with caution, because taking biopsy in animals differs from taking biopsy in human. In addition, taking bone core samples in human studies is done in different ways, some are taken vertically, and some are taken horizontally [30]. In this study trabecular thickness and percent of osteogenesis was higher in test group than control group, which is in accordance with the studies in which growth factors derives for increased bone regeneration from the patient’s own blood [31-35].

KutKut et al.8. suggested the positive effect of PRP and bone regeneration enhancement. Other similar results confirming to our findings are reported by Kassolis [36], Marx [37] and Anitua [22]. In a study done by Gürbüz et al., [38] PRGF was used for bone regeneration, and PRGF ineffectiveness was reported. In addition, other similar studies indicated the ineffectiveness of PRP in bone defects [39, 40], which was in contrary to the results of this research. Some studies indicate that a reason explaining this discrepancy can be various methods in PRGF production, (including system, different concentration of platelet and growth factors, time of application). Also, PRP effect is dose dependent, and is mainly effective in 9 to 6 times concentrations, while the higher concentrations have binding effects [38-43]. Based on the current study, a slight residual biomaterial inflammation is seen in test group compared to control group, which is an indication that allograft material is quickly absorbed and well tolerated. A similar result is observed in KutKut et al. studies8. Therefor this can be an indication that autologous blood-derived growth factors have accelerative role in allograft material absorption and its transformation to bone. According to findings of present study regarding biomaterial contact quality, in all the cases a direct contact and lack of connective tissue is reported which is in contrary to findings of Anitua et al [23], as they indicated that connective tissue exist between allograft material and bones. Considering vitality of newly formed bones, it seems that allograft material with and without platelet-rich plasma acts as a framework for normal osteogenesis, which is conforming to the results of studies done for autologous blood-derived growth factors [8, 42, 43]. In a study done by Toloue et al. the average residual allograft after application of calcium sulfate with freeze-dried bone allograft material is reported 2.5%, however, in current study the residual allograft is less than this value, which is probably because of absorption accelerative factor of PRGF in grafted region30. In this study DFDBA allograft was used as a useful substitute for autogenous allograft in patients with alveolar ridge defects. The results are in accordance with results of studies in which allogeneic bone material is used for augmentation of ridge defects and alveolar ridge preservation [12-18]. Since
autograft causes unwanted surgery trauma to other parts of the body, there is no doubt that, a suitable substitute with similar character that eliminates the need for allograft removal surgery will be beneficiary to both patient and physician. DFDBA is as osteoinductive allograft but FDBA is considered an osteoconductive allograft, however, based on laboratory researches, DFDBA has a higher potential for osteogenesis than FDBA. This will significantly increase vital bones compared to FDBA, therefore it is considered a preferred method, which is in accordance with studies in which DFDBA is used as bone allograft material for alveolar ridge regeneration.

The material used as barrier membrane in GBR should have properties such as tissue compliance, tissue integration, preventing invasion of adjacent host tissue cells, and simple clinical application. One of the drawbacks of bio-absorbable membranes is limited control over membrane absorption time as it supposed to be capable of maintaining its structural rigidity for a period time of over six months while it promptly undergoes decomposition by enzymes derived from macrophages and neutrophils and loses its rigidity. In current study, a pericardium absorbable allogeneic membrane with 0.2 to 0.6 mm thickness was used, therefore it advisable to perform a similar study using non-absorbable membrane to ensure that soft tissue is ineffective in guided osteo-regeneration process. One of the advantages of the current study is its humanistic nature that enables its finding to be generalized for other human samples.

**Conclusion:**

Based on the results of the study, the histomorphometric findings suggest that PRGF enhanced bone regeneration more than PRGF-free graft. Although no clinical and histological difference were found.

**Fig. 2:** Surgical procedures.

*Images histologic and histomorphometric in test group indicated:*
- Grade 1: inflammatory infiltrate (slight)
- area vital bone regeneration
- Score blood vessels: 2
- less residual biomaterial
Images histologic and histomorphometric in control group indicated:
- Grade 3: inflammatory infiltrate
- Area vital bone regeneration
- Score blood vessels: 1
- Greater residual biomaterial

REFERENCES


