

Original Article

Clinical, histological, and histomorphometrical comparison of CenoBone® with and without plasma rich in growth factor for edentulous ridge preservation in the dental sockets

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ABSTRACT

Background: The aim of this study was to compare the clinical, histological, and histomorphometrical outcomes of CenoBone® allograft with and without plasma rich in growth factor (PRGF) for the preservation of edentulous ridge in the dental sockets.

Materials and Methods: This study is experimental clinical trial that 14 dental sockets were included the sockets required ridge preservation followed by implant placement in the premolar and molar of the mandible. After extraction of the teeth, the CenoBone® allograft and PRGF were used in the test group and CenoBone® allograft was used alone in the control group. During the first stage of surgery and 5 months later, in the second stage of surgery (implant placement), the vertical changes of the ridge were measured. Furthermore, using Core-Biopsy in the second stage of surgery, criteria of histologic and histomorphometric were determined. Data were analyzed with *t*-test, Mann–Whitney *U*-test, and Fisher's exact test at the level of significance of $P < 0.05$.

Results: The mean trabecular thickness in the test group (52.18 ± 5.53) was significantly higher than that in the control group (41.53 ± 10.40) ($P = 0.344$). However, there were no significant differences in the mean values of vertical bone absorption, bone percentage, remaining biomaterials, inflammation, and blood vessels between the two groups. There was no case of foreign body reaction and the bone was vital in all the cases and in direct contact with the biomaterial.

Conclusion: Although CenoBone® allograft with PRGF was effective in some histomorphometric factors such as trabecular thickness, it did not lead to significant clinical changes.

Key Words: Allografts, dental implants, plasma, socket graft

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INTRODUCTION

Tooth extraction occurs when the tooth cannot be restored or maintained in a proper condition for long-term health, performance, or esthetics. Loss of teeth has a direct impact on the quality of life and

impairs the ability to chew, talk and, in some cases, socialize. After tooth extraction, bone resorption is a progressive and irreversible process that is well

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documented in authentic scientific studies. Alveolar bone resorption might reach 40% in height and 60% in width.^[1] Inadequate bone might endanger the implant treatment by impinging on the anatomic structure. Therefore, preserving the alveolar ridge is essential for the esthetic outcomes and proper insertion of the implant.^[2,3]

Protecting the alveolar ridge immediately after tooth extraction minimizes the resorption of the residual ridge and prevents soft and hard tissue collapse.^[4] The defects in the tooth extraction site, without the involvement of regeneration methods, lead to the formation of connective tissue fibrosis and do not fill with bone, especially when the supporting bone is lost after removing the tooth. Negative consequences of removing the tooth result in resorption of the bone and soft tissue around it, leaving an anatomic defect that requires augmentation treatments before implant placement.^[5] Maintaining the dimensions of the alveolar ridge after tooth extraction and provision of sufficient bone volume to stabilize the implant and achieve ideal prosthetic outcomes are functionally and esthetically critical factors in the tooth extraction site to prepare the area before placing the implant.^[6-8]

There are several methods for bone reconstruction, among which guided bone regeneration (GBR) has the best evidence for the treatment of localized bone defects. The use of GBR provides an area for the application of intraosseous implants in areas of the jaw that have an insufficient bone volume.^[9] The well-known gold standard method for bone grafting is the supply of autogenous bone from intraoral and extraoral sources; however, due to limitations such as the second osseous surgery problems, it has resulted in the selection of suitable bone replacement materials, such as allografts.^[10-14] Products produced from allogeneic sources are used in multiple surgical procedures due to bioavailability and bone remodeling capabilities in their bones.^[15]

The membranes are used in the GBR technique as the barrier for tissue growth and obtain better relationships. The two types of absorbable and nonabsorbent membranes are available. In order to eliminate the need for second surgery, absorbable types were introduced. These membranes improve the soft-tissue repair and integrate with the host tissue; in addition, when exposed to membranes, they quickly absorb and their microstructure is protected from contamination.^[16] The use of endogenous and

biologically active proteins for regenerative purposes has opened up new horizons for tissue regeneration.

In 1999, Anitua described a new technique for the preparation of platelet-rich plasma called plasma rich in growth factor (PRGF).^[17] This is a 100% autologous preparation that is rich in biological mediators to accelerate the hard and soft tissue regeneration. Plasma-derived adhesive molecules such as fibrinogen, fibronectin, vitronectin, and thrombospondin-1 act as a matrix or scaffold and are a precursor and platelet absorber. Platelets are a rich source of growth factors such as platelet-derived growth factor, transforming growth factor β , vascular endothelial growth factor, fibroblast growth factor, insulin-like growth factor, and granulocyte-macrophage colony-stimulating factor.^[18] In relation to PRGF in various studies, this substance might improve bone repair and result in proper restoration.^[18,19] It has been shown that the combined grafting material of PRGF with dental socket after extraction of the tooth is more favorable, and the epithelialization of the socket surface is favorable with PRGF. In addition, the use of PRGF in dental implants improves osseointegration, bone-implant contact, and soft tissue repair in the surgical site.^[20]

According to Jenabian and Poori and similar research, in the group in which growth factors and bone graft material were used, there were less inflammation and biomaterials remnants compared to the group in which the biomaterial was used alone. In the study group (growth factor + biomaterial), the biomaterial was rapidly absorbed and soon turned into bone.^[21] Available research on the use of bone allograft with autogenous growth factors to restore and protect the alveolar ridge shows positive results, but no definite conclusions have been drawn due to the limitations in this field.^[20] The aim of this study was to evaluate the effect of transplanting of CenoBone[®] allograft and Ceno membranes[®] in conjunction with PRGF to preserve the ridge for implant placement.

MATERIALS AND METHODS

This experimental clinical trial study was conducted with the code of ethics: IR.MUBABOL.HRI.REC 1397.236 and Code of IRCT: IRCT20100427003813N10 at the Iranian Center for Clinical Trials (IRCT).

This study was performed in the Department of Periodontics, Babol University of Medical Sciences. The samples consisted of 14 tooth sockets of

mandibular premolar and molar teeth that could not be preserved and had to be extracted. Patients who were healthy systemically, had good co-operation and proper oral hygiene were included in the study. Informed consent was taken from all patients before the beginning of the study.

Systemic conditions that affected the healing process such as diabetes, a history of alcoholism, immunological diseases, pregnancy, use of anticoagulants and immunosuppressive drugs, smoking, poor cooperation, periodontal disease and poor oral hygiene were the exclusion criteria. The tooth sockets were divided into two groups: Case and control.

In the case group, CenoBone® (Demineralized freeze dried bone allograft, made by Hamanand Saz Baft Tissue Regeneration Corporation, volume: 0.5cc, particle size: 150–2000µm) allograft material and PRGF were used and in the control group only CenoBone® allograft was used. In both groups, absorbable membranes (Ceno membrane: Made by Hamanand Saz Baft Tissue Regeneration Corporation, size: 15 mm × 20 mm, thickness: 0.2 × 0.6) and free gingival graft (FGG) were used and the effect of the PRGF was evaluated.

Clinical, histologic, and histomorphometric parameters were evaluated in the subjects. Clinical parameters included bone resorption on the distal, mesial, buccal, and lingual aspects.

Histomorphometric parameters include new bone formation, percentage bone trabecular thickness, and residual biomaterial percentage.

Histopathology, degree of inflammation, presence or absence of foreign body reaction, bone vitality, and biomaterial-bone contact and blood vessel count were measured.

Primary outcome

Determination of clinical, histologic, and histomorphometric comparisons of Ceno Bone® allograft with and without PRGF to preservation of edentulous ridge in the dental socket.

Secondary outcomes

Investigations on the number of blood vessels, vitality, and the absence of connective tissue between biomaterials and bone in the center of the socket cavity and how the trabecular thickness, bone resorption, and biomaterial remain in the center of the dental socket cavity.

First stage of surgery

Half an hour before surgery, 500 mg of amoxicillin and chlorhexidine mouthwash were administered.^[21] After anesthetic injection with 2% lidocaine, an envelope flap was prepared and the tooth was extracted atraumatically using a periosteal elevator [Figure 1]. The socket was irrigated with saline solution and granulation tissue was removed from the area. At this stage, the measurement of the socket wall was carried out at the mesial, distal, buccal, and lingual points by a prosthetic stent. In the case group, blood was taken from the patients before the surgery and PRGF was prepared. After tooth extraction, CenoBone® allograft was impregnated with PRGF and used to fill the tooth socket. The socket was filled up to the crest surface completely by the allograft. The Ceno membrane was impregnated with PRGF and placed on in the tooth socket so that 3 mm of socket in all parts to be taken [Figure 2]. Finally, FGG was placed on top of them and the flap was secured with a reverse cross-mattress suture [Figure 3]. All these steps were performed in the control group, too. However, in this group, PRGF was not used. At the end of the surgery, the patient was given 500 mg of amoxicillin three times a day (for 1 week) and chlorhexidine mouthwash twice a day (for 2 weeks).^[21]

Follow-up time was performed 2 weeks after surgery and then once a month for 5 months.



Figure 1: Atraumatic extraction.

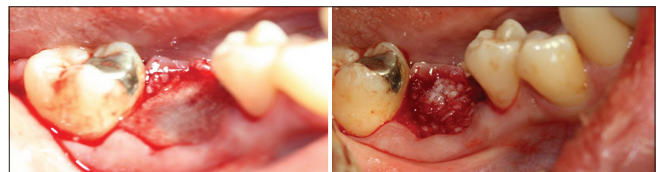


Figure 2: Placing Ceno bone® and Ceno membrane®.



Figure 3: Free gingival graft and suturing.

Second stage of surgery

After 5 months, the patient was recalled for the second stage of surgery (implant placement). Clinical evaluations were performed and panoramic and cone-beam computed tomography views were provided. Local anesthesia was injected into the area and a mucoperiosteal flap was reflected. Bone height was measured in the mesial, distal, buccal, and lingual aspects with the same stent and bone resorption was measured in these areas. From the center of the ridge (the placement of the implant), a vertical core of the bone was removed using a trephine (diameter: 3 mm and height: 8 mm) with irrigation at a bur speed of 1500 rpm [Figure 4]. The core biopsy was placed in 10% formalin to be sent to the pathology laboratory. The implant was then placed in the region according to the protocol and the cover screw was placed. The flap was sutured and antibiotics, analgesics and mouthwashes were re-administered. At this stage of surgery, there was no difference between the case and control groups.

Plasma rich in growth factor preparation

Blood samples were taken prior to surgery and transferred into 5 mL tubes with 8.3% sodium citrate as an anticoagulant. Then, the tubes were centrifuged for 8 min (480 g, 18000 rpm). As a result, blood was divided into the following layers:

1. Plasma has a small amount of growth factors or plasma poor in growth factors (PPGFs) in the upper part of the tube (1 mL)
2. Plasma is twice as much the usual growth factor or plasma with growth factors (PGFs) of 0.5 mL of the total volume of the tube
3. PRGF was 0.5 mL, above the red cell section in the tube
4. Red cell concentrate.

With a 1000- μ L pipette, PPGF was removed. The lowest plasma platelet count was in PPGF. PGF was also removed with a 500- μ L pipette. The red cell layer was removed by a thin layer of white cells (buffy coat) from the PPGF layer. This part was also isolated by a 500- μ L pipette and transferred into another tube containing 10% calcium chloride. For each 1 mL of PRGF, 50 μ L of 10% CaCl₂ were add.

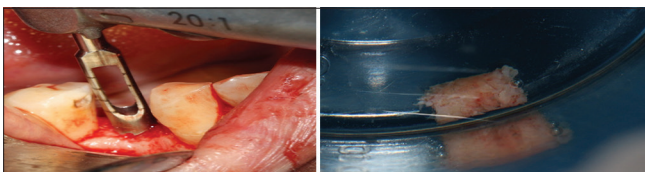


Figure 4: Trephine bur and harvesting bone core.

Clinical evaluation

Before first of surgery, the crown of the nonrestorative tooth was cut and the molding was done with alginate and a template was made on the cast. In this template, four points of mid-buccal, mid-lingual, and mesial and distal were pierced with bur.

Vertical measurement was done using casts, templates and 15 mm probes. Vertical measurements were performed at mesial, distal, buccal, and lingual points in the first surgery and second surgery. Spacing between the coronal guide stent with the midline of the socket wall in the buccal, lingual, mesial, and distal after extraction the tooth and 5 months later provided a clinical evaluation of the vertical ridge absorption.

Histopathologic evaluation

Core biopsy was kept in formalin 10% solution until complete fixation (7–10 days). Then, the crestal section of the core was marked with Indian ink and cores were placed in 10% nitric acid for 4–5 days until decalcification occurred. To neutralize the acid, samples were placed in 20% lithium bicarbonate solution. Finally, the bones were divided vertically in the antero-posterior direction. The incision edge representing the middle part of the bone was marked by Indian ink and the sample identification code was written. The samples were then placed in various concentrations of alcohol for serial dehydration, marked from the same part and placed in paraffin blocks. The paraffin blocks belonging to each bone sample were stacked into seven microscope slices and stained with hematoxylin-eosin and evaluated under a light microscope (Olympus BX41).

To examine each bone core, three microscope plates were used and in each plate, three microscopic fields were investigated. The mean of these data was used for each sample.

Histopathological evaluation consisted of histological and histomorphometric sections. Histologic evaluation consisted of (1) inflammation severity; (2) biomaterial–bone contact (presence or absence of connective tissue); (3) blood vessel counts; (4) bone vitality; and (5) foreign-body reaction.

Histomorphometric evaluation consisted of (1) trabecular bone thickness; (2) new bone area percentage; and (3) biomaterial area percentage.

The number of blood vessels in a microscopic field was evaluated at a magnification of $\times 10$ then they are evaluated at $\times 40$ magnification and scored:

- There were fewer than three blood vessels: 0
- Between 3 and 5 vessels: 1
- More than 5 blood vessels: 2.

Inflammation was categorized in five degrees:

Grade 0: The absence of inflammatory cells

Grade 1: Small and scattered (mild) inflammatory cells

Grade 2: The presence of 5–10 inflammatory cells (focal)

Grade 3: The presence of inflammatory 11–50 cells (focal)

Grade 4: Inflammatory cells with more than 50 focal lengths (severe inflammation).

Histomorphometric evaluation of bone

All the sections prepared from each biopsy sample were photographed by the DP12 camera under an Olympus microscope at $\times 40$ magnification and JPEG images were imported into the Motic plus software program. Then, the areas of bone were selected and the percentage of bone formed was calculated in terms of the total area of the image.

In the histomorphometric study, the thickness of bone trabeculae was determined in three degrees: [20]

Grade I: >60 microns (thick)

Grade II: Between 21 and 60 microns (moderate)

Grade III: Between 1 and 20 microns (thin).

Analysis of data

Data were analyzed using the SPSS software version 16 (SPSS, IBM Corp., Chicago, USA). The means and standard deviations of the variables were recorded. Data were analyzed using *t*-test and Mann–Whitney *U*-test. Statistical significant was set at $P \leq 0.05$.

RESULTS

Clinical findings

Vertical bone resorption in the distal, buccal, mesial, and lingual regions in the case and control groups is shown in Table 1. The results showed that vertical ridge resorption in the PRGF group (case) and the control group did not show a statistically significant difference.

Histomorphometric findings

The mean thickness of the trabecular bone, the area of bone formation, and the remaining biomaterial in

the case and control groups are shown in Table 2. According to the table, there was a significant difference in the thickness trabecular bone between the case and control groups ($P = 0.034$) [Figure 5]. However, the area of bone formation and the remaining biomaterial were not significantly different between the two groups.

Histologic findings

The mean blood vessel counts in the case and control groups were 2.47 ± 0.88 and 2.094 ± 1.67 , respectively, with no significant difference between the two groups ($P = 0.259$).

In the PRGF group, there were six samples with Grade 1 inflammation; only one sample exhibited Grade 2 inflammation.

Table 1: Vertical bone resorption in edentulous ridge in the case and control groups

Vertical bone resorption (mm)	Mean \pm SD		<i>P</i>
	Test group	Control group	
Mesial	0.61 \pm 0.35	0.65 \pm 0.32	0.902
Distal	0.54 \pm 0.39	0.41 \pm 0.47	0.620
Buccal	0.20 \pm 0.33	0.72 \pm 0.66	0.128
Lingual	0.15 \pm 0.61	0.31 \pm 0.34	0.318

SD: Standard deviation

Table 2: The mean thickness of trabecular bone, the area of bone formation and the remaining biomaterial in the case and control group

Variable	Mean \pm SD		<i>P</i>
	Test groups	Control groups	
Trabecular thickening (μ m)	52.18 \pm 5.53	41.53 \pm 10.40	0.034
Area of bone formation (%)	45.044 \pm 7.55	44.044 \pm 7.39	0.807
Remaining biomaterial (%)	2.61 \pm 4.76	4.014 \pm 6.27	0.53

SD: Standard deviation

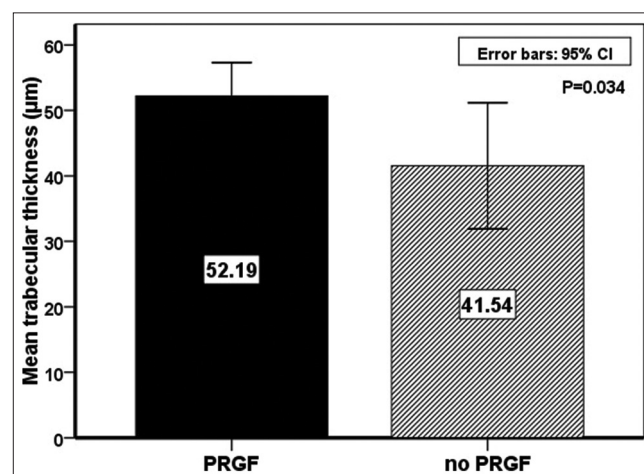


Figure 5: The mean thickness of trabecular bone in the case and control group.

There was no foreign-body reaction in any of the samples and bone was vital in all the samples; no connective tissue was seen at bone–biomaterial contact [Figure 6].

PRGF increased the thickness of trabecular but there was no significant difference in the number of blood vessels and inflammation between the two groups.

DISCUSSION

The aim of this study was to evaluate the clinical, histologic, and histomorphometric outcomes of CenoBone® allograft with and without PRGF in the preservation of edentulous ridge in tooth sockets.

In this study, vertical resorption of ridge in the mesial, distal, buccal, and lingual aspects was not significantly different between the case and control groups. Regarding histomorphometric analysis, only the thickness of bone trabeculae in the test group was significantly higher than that in the control group, but there were no significant difference between groups in the percentage of bone and the remaining biomaterials. There was no significant relationship between the number of blood vessels and the severity of inflammation in the test and control groups, and the bone in all the samples was vital in the two groups, and direct bone and biomaterial contact was observed in all the samples of the case and control groups. There was no foreign body reaction in any sample.

Despite the fact that a number of laboratory studies have shown positive outcomes with the use of PRGF in relation to osteoblasts and fibroblasts, clinical studies appear to not show significant outcomes.^[22,23]

As indicated, the two groups did not show a significant difference in clinical parameters (vertical ridge analysis), consistent with the results of a study by Jenabian and Poori,^[21] Kutkut *et al.*^[8] Samandari *et al.*^[24] in an animal study showed that PRGF was not

effective by ridge preservation after tooth extraction, which was in accordance with this study.

In the histomorphometric analysis, the thickness of bone trabeculae in the group of allograft biomaterial and PRGF showed more significant change than with the control group (allograft biomaterial alone), which is consistent with a study by Jenabian and Poori.^[21]

In other cases of histomorphometric analysis, including bone formation percentage and the residual biomaterial, no significant differences were found, which is different from the results of studies by Jenabian and Poori^[21] and Anitua *et al.*^[25]

In addition, the rate of residual biomaterial in our study was 2.61%, which is similar to the results of a study by Toloue *et al.*,^[9] who used allograft and calcium sulfate, indicating a lack of the effect of PRGF on the residual biomaterial in this study.

Some studies have shown the positive effects of PRGF on clinical findings and new bone formation, and some others have reported no such effects; such controversy between the results of different studies can be attributed to different PRGF preparation techniques and delivery times, and the effect of these autologous blood factors at certain concentrations. For example, some blood products, such as PRP, are effective at concentrations of 4–9 times, and can have inhibitory effects at higher concentrations,^[9,26-29] and this can also be true for PRGF. Furthermore, the time of the follow-up is different in studies on the effects of PRGF (from 3 months to 12 months) and this can also affect the study results.

Vitality of the bone in all the samples (test and control groups) indicates that the graft material, with PRGF or without PRGF, serves as a scaffold for osteogenesis.^[30,31]

The inflammation severity in the present study was similar to that in the study by Kutkut *et al.*,^[8] and in all the cases, inflammation was mild.

Contrary to the results of a study by Anitua *et al.* in relation to the presence of connective tissue between biomaterials and bone, in this study, the treatments performed in the test and control group did not result in any connective tissue between the biomaterials and bone.^[25]

Although the tissue healing index was not a goal in this study, at the time of suture removal, all subjects in the test group (PRGF) was shown better

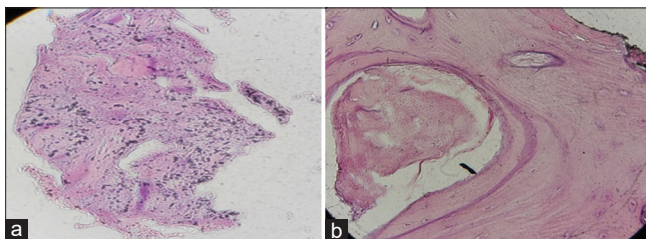


Figure 6: (a) Biomaterial and new trabeculae and blood vessels in PRGF group, (b) Biomaterial and new trabeculae in the control group. PRGF: Plasma rich in growth factor.

healing (tissue color, granulation tissue, bleeding during touch, red halo and...) in comparison with the control group. The membranes used in the GTR and GBR techniques should have tissue adaptation and space preservation properties, should be easy to use and should result in tissue integrity. One of the disadvantages of resorbable membranes used in the present study is membrane resorption control, which cannot maintain its structural strength for a long time. Therefore, it seems it is more helpful to use nonresorbable membranes in the process of bone regeneration.

CONCLUSION

The use of CenoBone® allograft with PRGF was effective in some histomorphometric factors, such as the thickness of the trabeculae, but it did not result in significant clinical changes.

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Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or nonfinancial in this article.

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