Histological Evaluation of the Effect of Concentrated Growth Factor on Bone Healing

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Objectives: The aim of this study was to evaluate the effects of concentrated growth factors (CGF) on the healing of peri-implant bone defects in an animal model.

Study Design: Twenty 4-month-old New Zealand White rabbits, each with an average weight of 3.5 kg, were used in this blinded, prospective, experimental study. Two implants were placed and 2 peri-impant defects were prepared in each rabbit tibia. Bone defects were created monocortically in the tibia of each rabbit using a trephine burr with a diameter of 8 mm. The implants were installed in each hole. The rabbits were divided into 4 groups: in group E, the defect was left empty; in group CGF, the defects were filled only with CGF; in group AB, the defects were filled with autogenous bone; and in group AB+CGF, the defects were filled with autogenous bone and CGF. The animals were euthanized at week 8 postimplantation. All implants from the 20 animals were fixed in 10% formalin and evaluated histomorphometrically.

Results: The mean defect area was highest in group E and lowest in group CGF+AB ($P < 0.05$). The area of the defect differed significantly between groups AB and CGF+AB ($P < 0.05$), but not between groups CGF and E. Implant-to-bone contact was lowest in group E. In the defect areas of groups CGF, AB and CGF+AB, a small amount of new bone formed around the implant.

Conclusions: In this animal model of a peri-implant bone defect, restoration was achieved using a combination of autogenous bone and CGF. Further studies are needed to determine the behavior of CGF when used in the repair of bone defects in humans.

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Implant therapy has become a successful and therefore popular treatment choice for the replacement of missing teeth. After tooth extraction, immediate placement of an implant into the fresh extraction socket yields successful clinical results. However, a gap often remains between the extraction socket wall and the implant. According to the literature, if the horizontal defect size is 1.5 mm or narrower, the mean bone-to-implant contact size is approximately 50% and a barrier membrane will be absent. In wider horizontal defects, a bone graft or guided bone regeneration can be considered,[1,2] although repair of the defect is not guaranteed. The graft materials commonly used to treat peri-implant defects include autografts, allografts, xenografts, and alloplastic grafts.[1] Of these, autogenous bone grafts are considered the gold standard because of their superior osteogenic and osteoinductive properties.[3] In addition, nonautogenous graft materials are costly and the healing times are long. Based on these considerations, studies on bone defect repair and healing continue.[4]

In recent years, concentrated platelets have been used in wound healing because of their high growth factor content. Among the preparations currently used for the regeneration and reconstruction of bone and connective tissues, including maxillofacial defects, are concentrated growth factors (CGF), platelet-rich plasma (PRP), platelet-derived growth factor, transforming growth factor beta, and platelet-rich fibrin (PRF).[4–9] Their use is aimed at shortening the interval between bone graft placement and implant insertion.[10,11]

Concentrated growth factor is produced by the centrifugation of venous blood, similar to the procedure used to obtain PRF, but the centrifugation speed differs.[10] Platelet-rich fibrin, a second-generation platelet concentrate, has been used alone or with graft materials to increase bone formation and to promote the healing of bone defects.[5] Thus, in the present study, we investigated the effectiveness of CGF, alone or in combination with autogenous bone, on bone regeneration in peri-implant defects.

METHODS

Animals and Materials

Twenty 4-month-old New Zealand White rabbits, each with an average weight of 3.5 kg, were used in this experimental study. The study protocol and experimental design were approved by the Animal Ethics Committee of Bulent Ecevit University and the animals were cared for in accordance with the guidelines of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes.

Surgical Method

General anaesthesia was induced by the intramuscular injection of 0.4 mL ketamine/kg and 0.3 mL xylazine kg. Preoperatively, 5 mL of intravenous blood was withdrawn from the rabbit ear veins. The blood samples were centrifuged to produce autologous CGF (MEDIFUGE; Silfradentsr, S. Sofia, Italy). The machine settings were as follows: 30 seconds acceleration, 2 minutes 2700 rpm, 40 minutes 2400 rpm, 40 minutes 2700 rpm, 30 minutes 3000 rpm and 36 seconds deceleration and stop. Centrifugation generates 3 blood layers: an upper layer containing platelet poor plasma, a middle layer consisting of CGF, and a lower layer made up of red blood cells. The rabbit tibias were shaved and disinfected and a peri-implant defect was created based on a previously described model.[12] An incision was made on the tibia and the periosteum was elevated from the bone. Two tibial bone defects, spaced 5 mm apart, were created monocortically using a trephine burr with a diameter of 8 mm (Fig. 1). An implant (diameter: 3.25 mm, length: 8.5 mm; BEGO GmbH & Co, Bremen, Germany) was then placed in each hole. The resulting artificial circumferential bone defects simulated alveolar defects with a circular gap of 2.37 mm. The rabbits were then divided into 4 groups: in group E, the defect was left empty; in group CGF, the defects were filled only with CGF; in group AB, the defects were filled with particulate autogenous bone harvested from the defect area with a trephine; and in group AB+CGF, the defects were filled with autogenous bone and CGF. The periosteum and skin were sutured using 3/0 silk. The rabbits received food and water until 8 weeks post-implantation. Like previous studies animals humanely killed 8 weeks after implantation.[12] All implants from the 20 animals were fixed in 10% formalin and evaluated histomorphometrically.

Histomorphometric Methods

The specimens were dehydrated in a graded ethanol series and embedded in methylmethacrylate-based resin (Technovit 7200 VLC; Kulzer & Co, Wehrheim, Germany). Undecalcified sections from the implants and surrounding bone were prepared. Each experimental site was sectioned longitudinally along the axis of the implant to yield serial sections 40-μm thick and stained with toluidine blue. All sections were analyzed in the histomorphometric evaluation, in which digital images from different areas of each histological slide were obtained at ×200 magnification using a motorized light microscope (Leica DM-4000B; Leica Microsystems, Wetzlar, Germany). Bone implant distances were calculated between the distance of the neck of implant screw and the point which bony structure was seen adjacent to the implant body (Fig. 2). The mean circumferential bone defect area was quantified (μm^2) using an image analysis program (Leica Q-Win Plus, ver. 3.5.1; Leica Microsystems, Heerbrugg, Switzerland) and the amount of new bone formation was calculated.

Statistical Analysis

The data were analyzed statistically using SPSS for Windows software (ver. 19.0; SPSS Inc, Chicago, IL). The Shapiro–Wilk test was used to determine the normal distribution of the data. Student *t* test (paired observations) was used to compare samples from the same animal. For unpaired observations, the values were compared using Welch and Tamhane T2 post hoc tests. A P value <0.05 was considered to indicate statistical significance.

FIGURE 1. The 2 peri-implant defects.

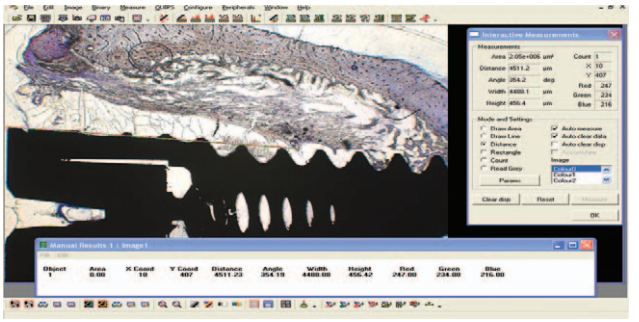

FIGURE 2. Bone implant distances were calculated between the distance of the neck of implant screw and the point which bony structure was seen adjacent to the implant body.

RESULTS

Animal

All animals well tolerated the surgery and none died unexpectedly during the postsurgical period. Wound dehiscence, wound infection, and abscess formation were not observed at any surgical site.

Histological Analysis

Based on observations of the undecalcified slides, stable fixation between the implant and cortical bone was achieved in all 4 groups. Implant-to-bone contact was less in group E. The fixation level was approximately one-third from the apical part of the implant in group E, but nearly two-thirds in groups CGF, AB, and CGF+AB. There was no contact between the new bone and the implant surface at the coronal parts of the implants in any group. New bone formation was higher in groups CGF, AB, and CGF+AB than in group E ($P<0.05$). In groups CGF, AB, and CGF+AB, a small amount of new bone formed around the implant (Fig. 3). Slight inflammatory cell infiltration, as well as loose and oedematous soft tissue, was observed at the coronal part of the implant surface at the defect area.

Histomorphometric

The mean defect area (mm^2) in each of the 4 groups is shown in Table 1. The mean area was highest in group E (4.72 ± 0.60) and lowest in group CGF+ABG (2.94 ± 0.36; $P<0.05$). The ABG group (3.65 ± 0.52) and CGF+ABG groups differed significantly ($P<0.05$), but the CGF (4.62 ± 0.61) and E groups did not.

The mean bone-to-implant contact distances (mm) are shown in Table 2. The highest distance was found in the group E (4.53 ± 0.37); however, there was no statistical significance observed between CGF group (4.35 ± 0.19) ($P>0.05$). The mean distance was 2.96 ± 0.4 in the CGF+ABG group, and 3.5 ± 0.31 in ABG group. The difference also was statistically significant ($P<0.05$).

DISCUSSION

The results of this histomorphometric study of bone defects surgically created around implants in rabbit tibias demonstrate the

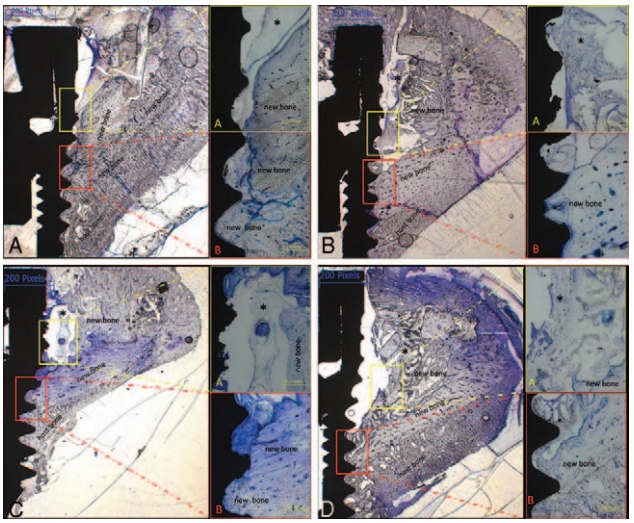

FIGURE 3. (A) Bone regeneration around the dental implant in the CGF+ autogenous bone group (toluidine blue staining, original magnification: ×200). (B) Bone regeneration around the dental implant in the CGF group (toluidine blue staining, original magnification: ×200). (C) Bone regeneration around the dental implant in the autogenous bone group (toluidine blue staining, original magnification: ×200). (D) Bone regeneration around the dental implant in the empty group (toluidine blue staining, original magnification: ×200). CGF, concentrated growth factors.

beneficial effect on bone regeneration of CGF when combined with autogenous bone grafts.

Rats and rabbits are frequently used in animal studies of newly formed bone in maxillofacial surgery because they can be more easily cared for than larger species and they are relatively inexpensive.[4] In our study, we used rabbits because the amount of blood obtained from rats is not sufficient for the preparation of CGF.

The surgical defect model used in this study is one that has been widely used elsewhere.[12–20] Our study evaluated new bone formation in peri-implant defect areas. So critical size defects in calvarium and alveolar region were not appropriate for study design. Because of our animal model tibia had adequate bone area for implant application. This defect model was used in previous publications.

The authors of the various reports differ in their opinions regarding the optimal size of the circumferential peri-implant defect. According to Boticelli et al[18] the width of the circumferential defect should be >1.25 mm. The other authors claimed that smaller defect sizes prevent an accurate evaluation of the effectiveness of the different grafting materials on bone regeneration. In our study, a 2.37-mm defect was created to prevent spontaneous healing.

Peri-implant defects are often treated with conventional bone regeneration methods, which rely on the use of membranes and graft

TABLE 1. Defect Area From Histomorphometric Analysis in Peri-Implant Defects

	Empty Defect	CGF	Autogenous Bone	CGF+ Autogenous Bone
Mean defect area	4.72 ± 0.60	4.62 ± 0.61	3.65 ± 0.52*,†	2.94 ± 0.36*,†

Data are expressed as the mean ± standard deviation.
CGF, concentrated growth factors.
*Statistically significant difference from empty defect and CGF groups ($P < 0.01$).
†Statistically significant difference between groups ($P = 0.018$).

TABLE 2. Distance in Peri-Implant Defects

	Empty Defect	CGF	Autogenous Bone	CGF+ Autogenous Bone
Mean distance	4.53 ± 0.37	4.35 ± 0.19	3.50 ± 0.31*,†	2.96 ± 0.4*,†

Data are expressed as the mean ± standard deviation.
CGF, concentrated growth factors.
*Statistically significant difference from empty defect and CGF groups ($P = 0.000$).
†Statistically significant difference between groups ($P = 0.022$).

materials. However, because membrane is expensive and carries a risk of disease transmission,[18] the use of growth factors has gained increasing acceptance. Platelet-rich plasma, which contains concentrated platelets and numerous growth factors, has been successfully used in the restoration of peri-implant defects,[18,21] but its production requires the addition of bovine thrombin and anticoagulants such that cross-contamination is possible.[22] Choukroun PRF is a second-generation platelet concentrate with several advantages over PRP: chemical additives such as calcium chloride and bovine thrombin are not needed, its production is less time-consuming, and its application is easier.[4,6,18] Platelet-rich fibrin leads to the formation of a robust three-dimensional fibrin matrix at the application site. This matrix allows for the slow release of the growth factors contained within PRF, which is not the case with PRP. Platelet-rich fibrin was previously used in a peri-implant defect model, where it was found to induce more and faster bone formation than in an unfilled control group.[18] Another study showed that, when used alone or in combination with hydroxyapatite/β-tricalcium phosphate, PRF increased bone regeneration in rabbit calvarial defects.[4]

As with PRF, CGF is produced by the centrifugation of venous blood, but the centrifugation speeds differ.[10] The production of CGF requires variable speeds to separate blood cells from fibrin-rich blocks, which are denser and contain a higher concentration of growth factors than PRF.[10,23,24] This results in a better regenerative capacity and greater versatility.[10,18] Because of the agglutination of fibrinogen factor VIII and thrombin, the fibrin clot in CGF is highly cohesive.[10,25] Also, CGF are gradually released.[26] In this study, CGF were tested alone and with autogenous bone graft for their ability to stimulate tibial bone regeneration in peri-implant defects.

Immediate implant installation has been recommended to prevent alveolar bone loss and to shorten the overall treatment period.[27] However, this is not possible if there are bone defects. Lee et al[18] used silk fibroin in conjunction with Choukroun PRF to treat peri-implant defects in rabbit tibias. Successful restoration of the defects was achieved by the application of PRF alone.[18] By contrast, in our study, there was no difference between the CGF and E groups. The difference can perhaps be explained by the fact that PRF releases growth factors for at least 7 days; this prolonged release may require a proper scaffold, provided in the study of Lee et al by the silk fibroin.

Other authors have also used CGF to accelerate bone healing.[10,23,26,28,29] Kim et al[26] used CGF, without any graft materials, in flapless transcrestal sinus augmentation. Autologous CGF were shown to be effective in other sinus augmentation procedures as well. For example, Sohn et al[28] tested CGF alone in sinus augmentation; the positive results were verified radiographically, clinically, and histologically. Those authors reported that CGF rapidly induced new bone formation in sinus augmentation, Together, these results recommend the use of CGF as a restoration material in bony defects. However, to the best of our knowledge, ours is the first study to evaluate CGF in the repair of peri-implant

defects. According to the histomorphometric results, significantly more new bone regeneration occurred in the CGF+AB group than in the AB group, but there was no difference between the CGF and E groups. Again, this may have been due to scaffold provided by the autogenous bone, which supported the prolonged release of growth factors from the CGF in an autogenous scaffold.

CONCLUSION

In conclusion, this study demonstrated that CGF, when used in combination with autogenous bone grafts, promotes bone regeneration in large defects (2.37-mm wide) around dental implants. In addition, our results, and those of other studies suggest that, in the repair of peri-implant defects, CGF is more effective when used in combination with a scaffold such as autogenous bone. However there may be different results between animal models and human applications. Further studies are needed to determine the behavior of CGF in the repair of critical size bone defects in humans and animals with long-term follow-up.

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