

A Case Report of Bilateral Mandibular Vertical Guided Bone Regeneration With and Without Bovine Thrombin/Calcium Chloride Activated Platelet-Rich Plasma

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One patient with a bilateral vertical defect was treated on one side with guided bone regeneration (GBR) and an autologous bone graft and on the contralateral side with the addition of platelet-rich plasma (PRP). At the 6-month reentry, clinically and radiographically enough bone width and height were present to allow implant placement in both sites. At the same time point, at the histologic level, no differences were noticeable. Similar results were obtained in this case in vertical bone regeneration with and without bovine thrombin/calcium chloride activated PRP applied to GBR techniques.

Key Words: *guided bone regeneration, platelet-rich plasma, bone augmentation, implants, bone graft, autologous*

INTRODUCTION

Deficiencies of the ridge have been treated in many ways to restore the original anatomy and improve function and esthetics of the fixed prosthesis. The different modalities include soft and hard tissue ridge augmentation, with or without guided bone regeneration (GBR).¹ Platelet-rich plasma (PRP) is an autologous source of platelet-derived growth factors,

which may be easily obtained by sequestering and concentrating platelets using gradient density centrifugation.² Because these factors play a critical role in cell proliferation, bone remodeling, and angiogenesis,^{3,4} increasing their concentration in the microenvironment may eventually convey a significant enhancement in the initial phase of bone regeneration. This article reports a single case of bilateral mandibular ridge defect treated according to the principle of GBR and autologous bone graft with and without PRP.

MATERIALS AND METHODS

The patient was a medically healthy 37-year-old white man with no underlying systemic

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disease, as assessed by medical history screening. The patient had a diagnosis of generalized chronic periodontitis with localized aggressive sites and was treated accordingly. Periodontal treatment consisted of oral hygiene instructions, scaling, and root planning, plus the extraction of the upper right second molar, which had probing depth up to 13 mm; class II furcation in the buccal, mesial, and distal furcation; and mobility of III; therefore, it was considered hopeless.

After 6 weeks, the patient was reevaluated and treated with an apically positioned flap in the maxillary posterior sextants. At the end of active periodontal treatment the patient was considered periodontally stable and enrolled in an active maintenance program. At that time the patient was considered eligible for reconstructive therapy. He exhibited good oral hygiene, had ability to maintain periodontal health, and had no contraindications for dental treatment. Informed consent was obtained before treatment.

Based on clinical and radiographic findings, the patient had a bilateral ridge defect class III according to the Siebert classification.⁵ As seen by computerized tomography (CT) scan analysis, the vertical defect was such that placement of implants was not possible without previous ridge augmentation. The patient chose to have the missing teeth replaced by endosseous implants, including preimplant bone augmentation procedures. At the time of surgery 50 mL of blood was drawn from the patient in a sterile test tube containing anticoagulant and processed in a PRP machine (Implant Innovation Inc, West Palm Beach, Fla) according to the manufacturer's instructions.

Surgical technique

The patient was premedicated with amoxicillin and 1 g twice a day at the beginning of the day of surgery and continuing for 1 week. The 2 sites were treated the same day. After

administration of an appropriate local anesthetic consisting of mandibular nerve block and local infiltrations with lidocaine 2% with epinephrine 1:100 000 for a total of 4 carpules, an initial incision was made on the midcrest. Vertical releasing incisions were placed at the mesial and distal line angles of the approximating teeth at a reasonable distance from the anticipated edges of the membrane. On the buccal side, a full-thickness flap was elevated up to the mucogingival junction and continued as a partial-thickness flap. The underlying periosteum was then reflected to expose the defect. On the lingual side, a full-thickness flap was raised in the beginning, and then at the base of the flap a partial-thickness incision was made to release it coronally. This partial-thickness release was made well above the milo-hyoideum attachment. The above incisions and flaps were raised to allow eventual coronal displacement of both the lingual and buccal flaps in order to achieve a tension-free primary closure over the vertically augmented area.

The ridge was extremely resorbed in both the apicocoronal and buccolingual dimension as estimated on the CT scan evaluation (Figure 1a). After exposure, the ridge was thoroughly debrided, and its status was recorded by intraoral photography. Intra-marrow penetration to allow blood perfusion of the recipient site of the graft was achieved with a small round bur. Two stainless steel screws, 5–7 mm long, were positioned on the ridge to ensure space maintenance under the membrane on one side (Figure 2a). The graft material consisted of approximately 1 mL of autogenous material harvested from a contiguous area to the one to be treated by a scraping instrument (MX Grafter, Maxilon Laboratories, Inc, Hollis, NH).

At the test (autologous + activated PRP) side, once the recipient site was prepared, calcium chloride and bovine thrombin were

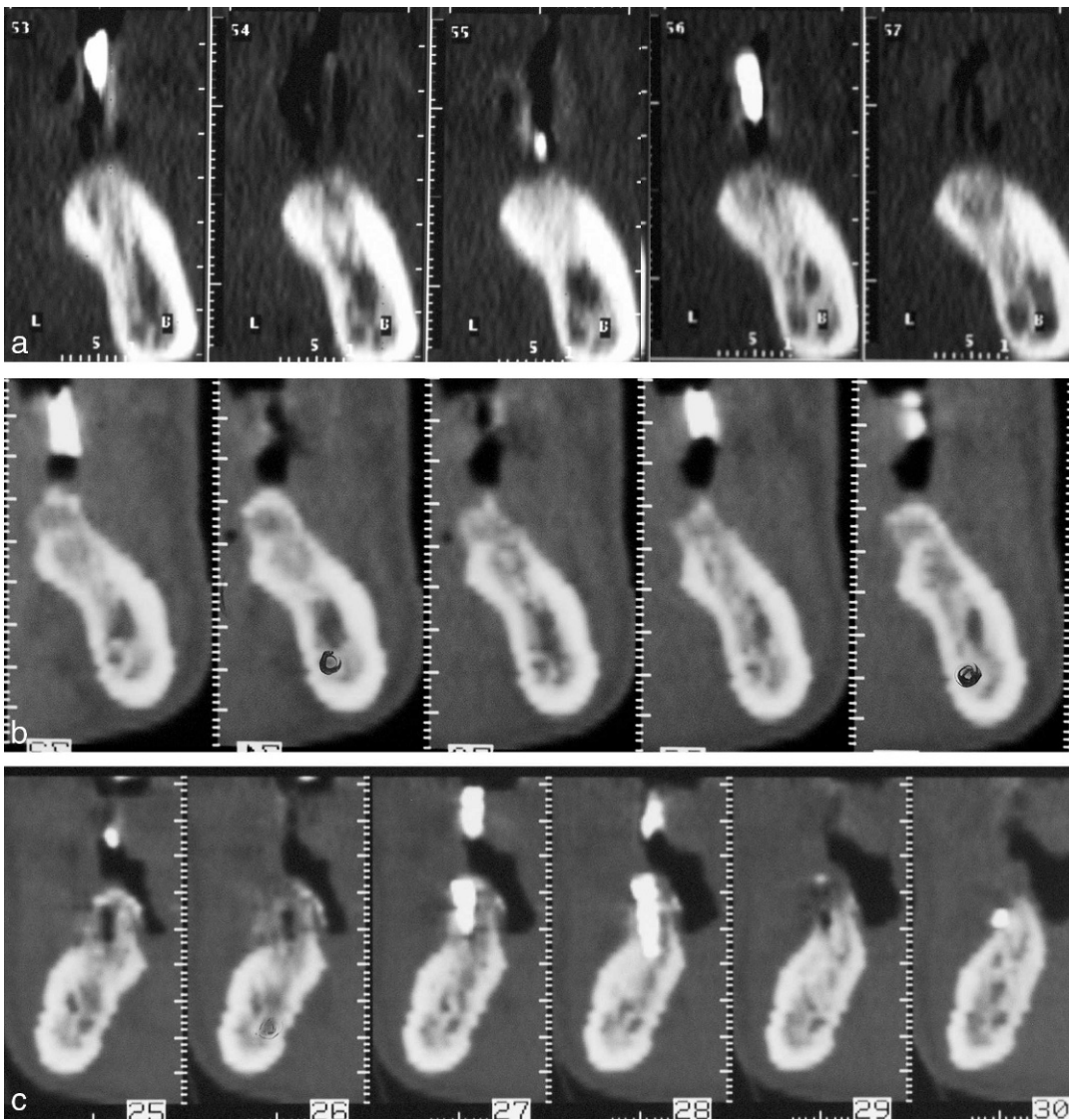
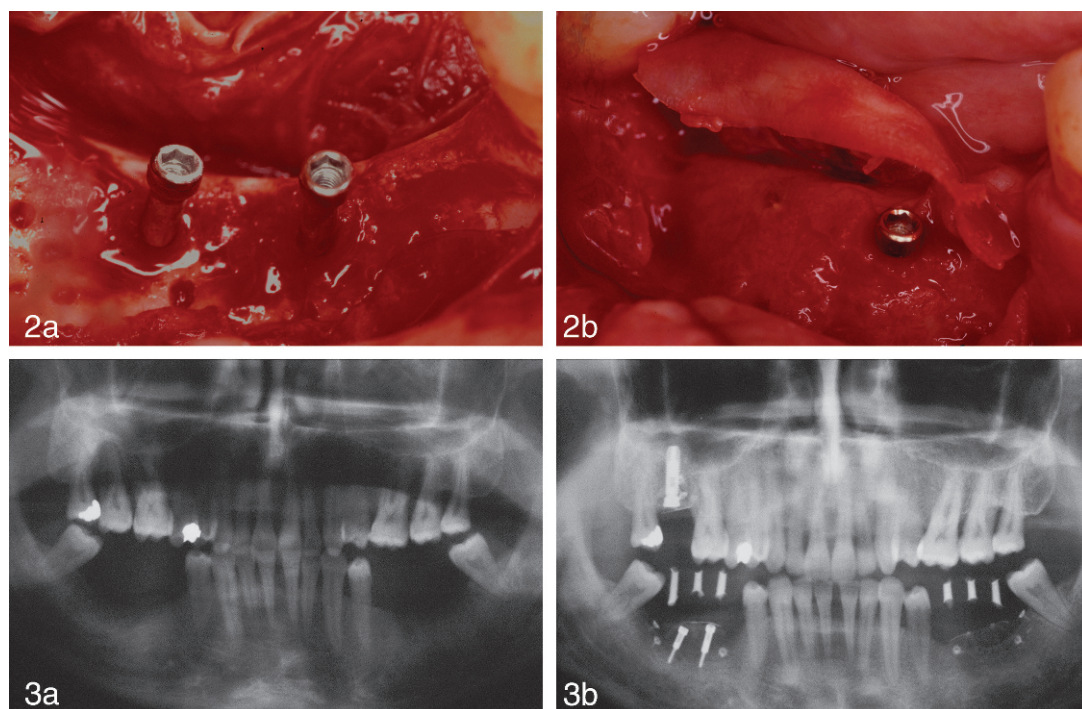


FIGURE 1. Computerized tomography scan. (a) Preoperative view showing insufficient bone in the apicocoronary and buccolingual dimension to place implants according to the prosthetic needs. (b) Postoperative view showing a robust bone regeneration of about 4 to 5 mm in the area corresponding to the radiopaque marks on the surgical stent. (c) At the contralateral side new bone formation is noticeable around the tenting stainless steel screw placed for space maintenance under a nonresorbable membrane.

slowly added to the PRP to begin platelet aggregation and activation, and this mixture was then added to the bone graft. The graft material was then easily placed in the defect in different layers. A nonresorbable expanded polytetrafluoroethylene (e-PTFE) titanium-reinforced membrane (GoreTex, Flagstaff, Ariz) of suitable size was trimmed and positioned over the defect, extending at least 2 mm in all directions onto sound bone. Care was taken to avoid contact with

adjacent teeth. The membrane was then fixed to the bone surface with titanium fixation pins 3 mm long (Ace Surgical, Brockton, Mass).

A tension-free primary closure of the flaps was achieved with nonresorbable, interrupted e-PTFE sutures. Postsurgical care included completion of the antibiotic cycle, nonsteroidal analgesic as needed and chlorhexidine gluconate 0.2% twice daily (Dentosan, Pagni, Florence, Italy) for 2 weeks to



FIGURES 2 AND 3. FIGURE 2. (a) Clinical view of the edentulous ridge with 2 stainless steel screws in place before ridge augmentation. (b) Clinical view of the same area showing the screws completely covered by the newly regenerated bone. **FIGURE 3.** Panorex view. (a) Preoperative view showing contralateral edentulous area in the mandible to be treated with vertical ridge augmentation before implant placement. (b) Follow-up panorex 6 months after contralateral augmentation: the left side is treated with guided bone regeneration and autologous bone graft, the right side in the identical manner with additional platelet-rich plasma.

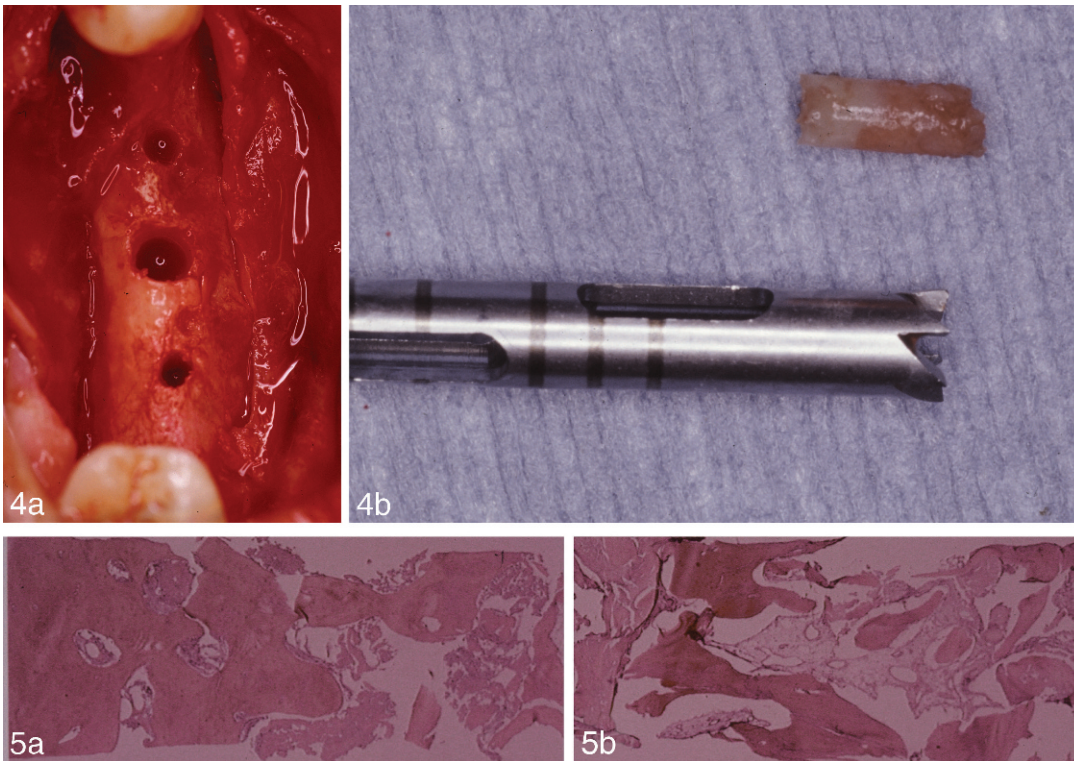
enhance plaque control. Sutures were removed 2 weeks after surgery. The membrane was kept in place until the time of implants placement, at 24 weeks. Healing was normal and unremarkable.

Bone-core sampling and histology

Endosseous implant fixtures were inserted 6 months after the grafting procedure at both test and control sites. At the time of implant surgery, the patients received an appropriate local anesthetic followed by elevation of a full-thickness mucoperiosteal flap. Augmentation of ridge height and width and localization of the grafted sites were established intraoperatively by comparison with initial photographs and radiographs (Figures 1b, 2b, and 3).

At the time of implant positioning, a surgical trephine of 2 mm ID (Implant Innova-

tion) was used to prepare the implant site and at the same time to harvest a core biopsy (Figure 4a and b). The implant fixtures were then inserted and the flaps were repositioned and sutured. The 2 samples, approximately 4 to 5 mm long, were immediately fixed in 4% formaldehyde (freshly made from paraformaldehyde) in 0.1 M phosphate buffer, pH 7.2. After washing in phosphate buffered saline, samples were decalcified for 3 hours in a standard solution of ethylenediaminetetraacetic acid disodium, potassium sodium tartrate, and diluted hydrogen chloride in distilled water, pH <1 (RDO solution; Du Page Kinetic Laboratories, Inc, Downers Grove, Ill), post-fixed in 4% formaldehyde for 2 hours and routinely embedded in paraffin. Sections 5 μ m thick were stained with hematoxylin and eosin and examined under transmitted and polarized light microscopy.⁶



FIGURES 4 AND 5. **FIGURE 4.** (a) Implant site preparation. The middle implant site is prepared with a surgical trephine of 2 mm internal and 3 mm external diameter to allow core harvesting. (b) Core bone biopsy of approximately 4–5 mm obtained with the surgical trephine bur. **FIGURE 5.** Histologic evaluation (hematoxylin and eosin). (a) Platelet-rich plasma (PRP) side. (b) Side without PRP. Representative histologic view under transmitted light microscopy. Similar features were observed in all the sections enclosed in this report. The newly formed bone is recognizable for the presence of osteocyte containing lacunae and for the woven structure. The intertrabecular spaces exhibit a mild degree of fibrosis but no features of inflammatory reaction.

RESULTS

At the time of implant insertion, clinical and radiographic evaluation revealed appropriate bone regeneration in the test and control sites (Figures 1, 2, and 3). At the histologic level, decalcified sections clearly showed graft particles surrounded by intimately apposed newly formed bone (Figure 5). Distinct cement lines were frequently evident at the interface between graft material and the newly formed bone. Osteocyte-containing lacunae were easily recognized within the new bone. The intertrabecular spaces exhibited a mild degree of fibrosis without signs of inflammatory reaction. Notably, a lack of fibrous encapsulation was evident in all the samples. Polarized light microscopy revealed a predominant woven

rather than lamellar structure of the newly formed bone.

DISCUSSION

Bone resorptive patterns may prevent the placement of endosseous implants. Many techniques have been described to create a more favorable surgical site for implant placement, ranging from socket preservation⁷ to maxillary sinus elevation⁸ and horizontal^{9–11} and vertical^{12–14} ridge augmentation. Bone grafting may be accomplished through the use of autogenous grafts,^{15,16} allografts,¹⁷ xenograft,¹⁸ or alloplastic material.¹⁹ As each graft material features specific advantages and limitations, loading the grafts with osteoinductive

agents (ie, growth factors) has been proposed to enhance new bone formation.²⁰ Even though attractive in terms of results and risk-benefit ratio, the use of growth factors and their handling in a private dental setting is undeniably complex and unfavorable in terms of cost-effectiveness. Recently, it has been shown that adding PRP to cancellous bone grafts to repair extensive mandibular defects resulted in a faster maturation rate and a greater bone density than in grafts without PRP.^{1,2} These results have been related to the availability of a higher concentration of platelets (more than 3 times the one in the original blood), which are a well-known source of growth factors, including platelet-derived endothelial cell growth factor and transforming growth factor beta. Because these factors are known to play a critical role in cell proliferation, and angiogenesis,^{3,4} the increase of their concentration in the microenvironment can explain both the faster maturation rate and the greater bone density in grafts implanted with PRP.² Recently, a systematic review concluded that evidence for beneficial effects of PRP in sinus elevation appeared to be weak and no conclusions can be drawn about other applications of PRP in dentistry.²¹

Lately it has been suggested that massive bovine thrombin addition may interfere with the 3-dimensional fibrin architecture,²² and an alternative slow polymerization, without chemical addition, may generate a fibrin network very similar to the natural one. Such a network may lead to a more efficient bone regeneration.²²

CONCLUSIONS

In this patient, 6 months after bone grafting the mandibular bilateral defects were regenerated. Clinical, radiographic, and histologic results showed that bone volume and maturation could allow implant placement

in both treated areas. The results were in fact comparable in both sites at the time of implant placement. This case report does not address whether the PRP-treated side reached maturation earlier or whether activation of the PRP with thrombin altered the availability of growth factors in the graft. It can be concluded that GBR is a viable method to restore vertically deficient bony ridge with or without addition of bovine thrombin/calcium chloride activated PRP.

ABBREVIATIONS

CT: computerized tomography
e-PTFE: expanded polytetrafluoroethylene
GBR: guided bone regeneration
PRP: platelet-rich plasma

REFERENCES

1. Brugnami F, Caleffi C. Prosthetically driven implant placement. How to achieve the appropriate implant site development. *Keio J Med.* 2005;54:172-178.
2. Marx RE, Carlson EC, Eichstaedt RM, Schimmele SR, Strauss JE, Georgeff KG. Platelet-rich plasma: growth factor enhancement for bone grafting procedures. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 1998;85:638-646.
3. Miyazono K, Takaku F. Platelet-derived growth factors. *Blood Rev.* 1989;3:269-276.
4. Bonewald LF, Mundy GR. Role of transforming growth factor-beta in bone remodeling. *Clin Orthop.* 1990;250:261-276.
5. Seibert JS. Reconstruction of deformed, partially edentulous ridges using full thickness onlay grafts: I. Technique and wound healing. *Compend Contin Educ Gen Dent.* 1983;4:437-453.
6. Bianco P, Ponzani A, Bonucci E. Basic and "special" stains for plastic sections in bone marrow histopathology, with special reference to May-Grünwald-Giemsa and enzyme histochemistry. *Basic Appl Histochem.* 1984;28:265-279.
7. Brugnami F, Then P, Moroi H, Leone C. Histologic evaluation of human extraction sockets treated with demineralized freeze-dried bone allograft (DFDBA) and a cell occlusive membrane. *J Periodontol.* 1996;67:821-825.
8. Boyne PJ, James RA. Grafting of the maxillary sinus floor with autogenous marrow and bone. *J Oral Surg.* 1980;38:613-616.
9. Brugnami F, Then P, Moroi H, Kabani S, Leone WC. Guided bone regeneration with DFDBA prior to implant placement. Clinical evaluation and histologic evidence of osteoblastic and osteoclastic activities in demineralized freeze-dried bone allograft. *Int J Periodont Restorative Dent.* 1999;19:259-267.

10. Nevins M, Mellonig JT. Enhancement of the damaged edentulous ridge to receive dental implants. A combination of allograft and the Gore-Tex membrane. *Int J Periodont Restorative Dent.* 1992;12:96–111.
11. Buser D, Dula K, Heso D, Hirt HP, Belser UC. Localized ridge augmentation with autografts and barrier membranes. *Periodontol 2000.* 1999;19:161–163.
12. Tinti C, Parma-Benfenati S, Manfrini F. Space-making metal structures for nonresorbable membranes in guided bone regeneration around implants. Two case reports. *Int J Periodont Restorative Dent.* 1997;17:53–61.
13. Tinti C, Parma-Benfenati S. Vertical ridge augmentation: surgical protocol and retrospective evaluation of 48 consecutively inserted implants. *Int J Periodont Restorative Dent.* 1998;18:434–443.
14. Simion M, Jovanovic SA, Trisi P, Scarano A, Piattelli A. Vertical ridge augmentation around dental implants using a membrane technique and autogenous bone or allografts in humans. *Int J Periodont Restorative Dent.* 1998;18:8–23.
15. Astrand P. Onlay bone grafts to the mandible In: Worthington P, Branemark PI, eds. *Advanced Osseointegration Surgery.* Chicago, Ill: Quintessence; 1992:123–128.
16. Riediger D. Restoration of masticatory function by microsurgically revascularized iliac crest bone grafts using endosseous implants. *Plast Reconstr Surg.* 1988;81:861–877.
17. Nevins M, Mellonig JT. Enhancement of the damaged edentulous ridge to receive dental implants: a combination of allograft and the Gore-Tex membrane. *Int J Periodont Restorative Dent.* 1992;12:96–111.
18. Froum SJ, Tarnow DP, Wallace SS, Rohrer MD, Cho SC. Sinus floor elevation using an organic bovine bone matrix (OsteoGraf/N) with and without autogenous bone: a clinical, histologic, radiographic, and histomorphometric analysis. Part 2 of an ongoing prospective study. *Int J Periodont Restorative Dent.* 1998;18:528–543.
19. Masters DH. Bone and bone substitutes. *CDA J.* 1988;16:56–65.
20. Louise FR, Borghetti AF. New developments in synthetic bone replacement materials. *Curr Opin Dent.* 1992;2:97–103.
21. Plachokova AS, Nikolidakis D, Mulder J, Jansen JA, Creugers NH. Effect of platelet-rich plasma on bone regeneration in dentistry: a systematic review. *Clin Oral Implants Res.* 2008;19:539–545.
22. Dohan D, Choukroun J, Diss A, et al. Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part I: technological concepts and evolution. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2006;101:e37–e44.