

# A Novel Drilling Procedure and Subsequent Bone Autograft Preparation: A Technical Note

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**Purpose:** To describe a new drilling system that allows the surgeon to obtain autologous living bone that, when associated with a plasma rich in growth factors (PRGF), can be used in bone grafting. **Materials and Methods:** Bone particles collected using both conventional and new drilling systems were analyzed by means of optic and electronic microscopy in 10 patients. Blood was collected from 43 volunteers and used to prepare PRGF. Quantitative aspects of the PRGF, including number of platelets and concentration of growth factors (insulin growth factor [IGF-I], transforming growth factor [TGF- $\beta$ 1], platelet-derived growth factor [PDGF-AB], vascular endothelial growth factor [VEGF], hepatocyte growth factor [HGF], and epidermal growth factor [EGF]) were assessed. A demonstrative case study was presented. **Results:** Microscopic examination showed that the bone structure and the presence of living cells in the bone chips were conserved in all samples obtained from drilling at low speed, whereas material obtained by conventional drilling did not maintain these qualities. Mean counts for TGF- $\beta$ 1 ( $55.27 \pm 16.23$  ng/mL), PDGF-AB ( $27.96 \pm 12.13$  ng/mL), VEGF ( $421.09 \pm 399.0$  pg/mL), EGF ( $455.49 \pm 210.04$  pg/mL), and HGF ( $605.70 \pm 269.20$  pg/mL) were significantly correlated with the number of platelets ( $590,000 \pm 197,000$  platelets/ $\mu$ L;  $P < .05$ ). **Discussion and Conclusion:** The new drilling procedure was developed based on biologic criteria. The method may reduce damage to the host tissue and can be used to obtain a mass of living bone for subsequent grafting in association with autologous growth factors. This new procedure may present new possibilities for enhanced bone healing and needs to be evaluated in a clinical trial. (Technical Note) INT J ORAL MAXILLOFAC IMPLANTS 2007;22:138-145

**Key words:** autografts, dental implants, low-speed drills, preparation rich in growth factors

A successful implant treatment protocol has evolved as a consequence of the introduction of new concepts into surgical practice. It is well known that success in implant dentistry depends on several parameters that might be improved considering both biologic and mechanical criteria.<sup>1,2</sup> When surgical techniques are undertaken with careful attention to detail, the invasiveness of the procedure can be limited to the area where bone regeneration is needed.

Implantation techniques currently in use involve drilling at speeds of 1,000 to 1,500 rpm to prepare potential recipient sites. The mechanical and thermal

damage to the tissue surrounding the implant during drilling could have a destructive effect on the initial state of the cavity housing the implant.<sup>3,4</sup>

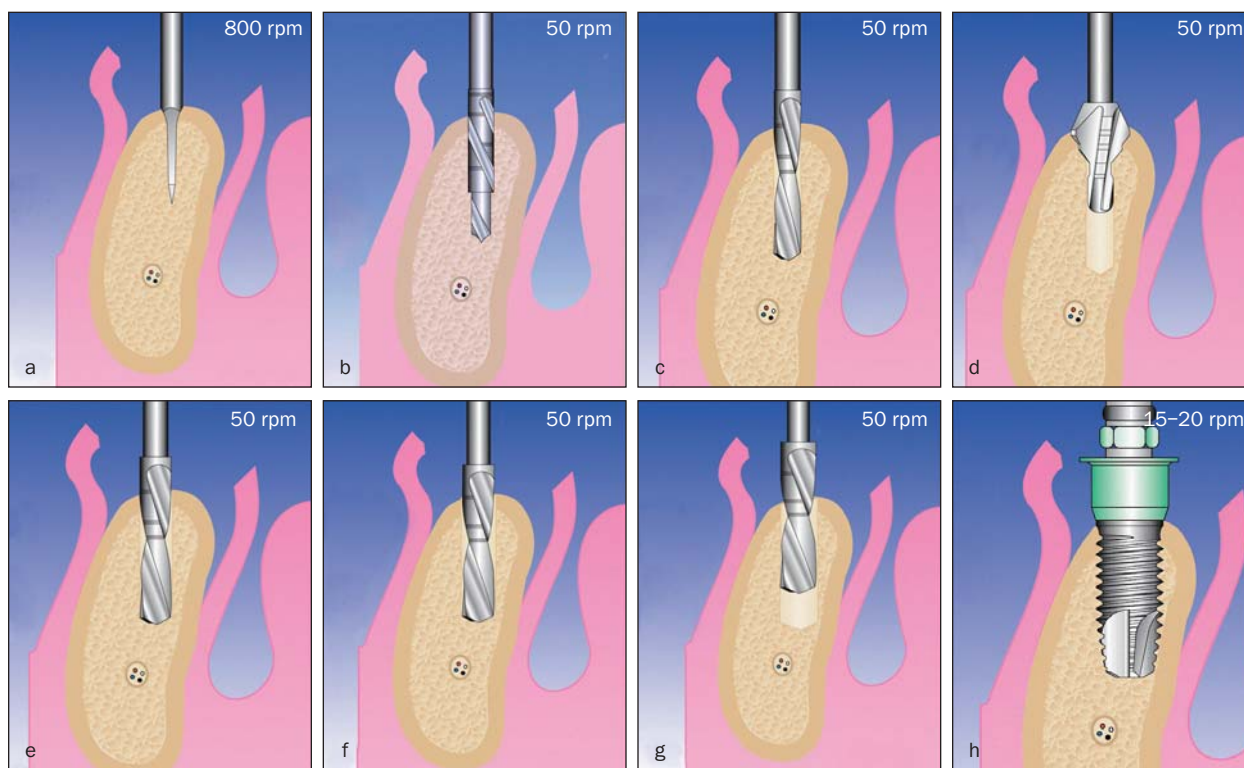
As a consequence, endogenous factors localized in bone extracellular matrix having a key role in the success of processes such as bone regeneration and bone-implant integration may be damaged. The use of autologous platelet-rich clots in dental implant surgery has broad use as an extended practical procedure by dental and maxillofacial surgeons as well as in other medical fields.<sup>5</sup> The authors' laboratory has developed a particular system for obtaining a preparation rich in growth factors (PRGF), and its advantageous effects have been documented in several fields, including implant dentistry and arthroscopic and orthopedic surgery.<sup>6-9</sup> The use of PRGF can accelerate bone regeneration after tooth extraction and around implants.<sup>10</sup> Another important benefit of the use of autologous platelet-rich plasma (PRP) clots is that it holds bone graft particles together.<sup>11</sup>

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**Fig 1** An innovative drilling procedure designed to preserve the peri-implant tissue. (a) The tip of the initial drill is very sharp. This drill rotates at 800 rpm with irrigation. (b to d) The pilot drill (b), the countersink drill (d), and (c; e to g) twist-type drills of different diameters (2.5 to 3.8 mm) are shown. Except for the initial drill, all drills are used at 50 rpm without irrigation. (h) The implant is placed at 15 to 20 rpm without irrigation.

The purpose of this article is to introduce an innovative alternative to conventional drilling procedures that maintains host bone in the best biologic conditions for receiving the implant. Additionally, bone collected by this procedure has been shown by means of optic and electronic microscopy to be living bone. This is particularly important because clinically autogenous bone has been reported to be the ideal defect filler.<sup>12</sup> Bone collected by this procedure may be easier to manipulate than bone collected by other means and can provide adhesive and signaling proteins involved in bone repair.

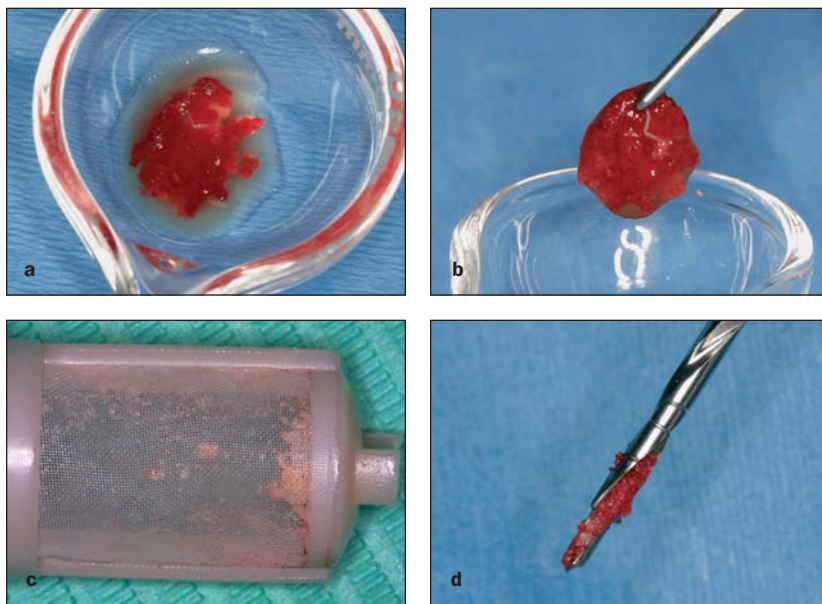
## MATERIALS AND METHODS

### Procedures

The original drill procedure and drill tools used in this article are from Biotechnology Institute, Vitoria, Spain (patent pending). As the drilling procedure must be adapted to the characteristics of the specific area to be drilled, the cortical layer is drilled with a very sharp design drill at a speed of approximately 800 rpm.

Serum irrigation needs to be applied during this phase. In the next steps the entire cavity (alveolus) housing the implant is formed, preserving the biologic properties of the host tissue as much as possible. To accomplish this, drills of various shapes and sizes are used (as shown in Fig 1). This process is carried out at low speeds (20 to 80 rpm) without irrigation. Eventually the countersinking phase is also carried out at low speeds of 20 to 80 rpm, without saline irrigation. The bone is collected directly from the drill. The threads of the twist drills have a retentive design (deep grooves) that enables the storage and subsequent retrieval of displaced tissue or cut trabecular bone. Drilling pressure should be kept under control; when the deposited bone offers resistance, it should be removed.

*Autograft Preparation.* After the drill bit is removed from the neo-alveolus, a perioste or another instrument is used to extract the tissue from the twist drills. The tissue is deposited in a small container made of glass or a similar sterile material. Thus tissue particles are collected directly, without the use of suction filters or additional tools. A composite



**Figs 2a and 2b** Autograft preparation with bone particles obtained during low-speed drilling.

**Figs 2c and 2d** Illustration of macroscopic differences in bone chips obtained using the 2 procedures: (c) conventional drilling and (d) the low-speed drilling procedure.

graft is obtained by mixing bone particles with PRGF. To prepare the PRGF, peripheral blood is obtained by venipuncture (minimum 10 mL) directly into 3.8% (wt/vol) sodium citrate (1 vol:9 vol). The blood is centrifuged at 460 g for 8 minutes at room temperature, and the plasma fraction located just above the sedimented red cells, not including the buffy coat, is collected and deposited in a glass dish. Calcium chloride is added to initiate clotting, and the developing clot includes the collected bone particles (Figs 2a and 2b). The mixture is used to fill postextraction sites or added around titanium implants for the over-correction of defects.

In order to prepare a fibrin membrane, the milliliter of the plasma fraction located at the top of the tubes was transferred to a glass bowl. Calcium chloride is added, and the mixture is incubated at 37°C for 20 to 25 minutes; during clot retraction, a fibrin membrane in the shape of the bowl is formed.

### Drill Temperatures

Pig mandibles were utilized to provide cortical bone. Two pilot drills (1.8/2.5 mm) and a 2.5-mm twist drill were used to examine the temperatures generated at the tip of the drill after perforating 10 mm deep using a conventional motorized dental handpiece (W&H, Bürmoos, Austria). First, a very sharp drill was used at 800 rpm with saline irrigation. Then, a pilot drill followed by a twist drill was used at 50 rpm to expand the hole. The temperature at the tip of each drill was recorded using a digital thermometer (Carlo Gavazzi PAN 133 Oregon Scientific, Tualatin, OR). Ten cavities were created following this procedure, and mean temperatures were calculated for each drill.

### Evaluation of Collected Bone

Ten patients agreed to participate in the study and gave their written informed consent. Both high- and low-speed drilling procedures were performed in opposite dental quadrants in the same patient. A conventional drilling method at 1,200 rpm with external irrigation was employed in the left dental quadrant. An aspirator equipped with a filter was used to collect all the bone particles (Fig 2c). Another aspirator was used to evacuate blood and saliva. Collected tissue was divided into 2 portions for evaluation by light microscopy and ultrastructural analysis. Low-speed drilling was performed in the right dental quadrant as described. Displaced bone tissue was recovered from the specially designed drill bits (Fig 2d) and divided into 2 portions for light and electron microscopic analysis.

Samples from both dental quadrants were processed by blinded examiners in an identical manner. Briefly, collected bone particles were fixed in 10% buffered formalin and decalcified in nitric acid. After inclusion in paraffin wax, the samples were sliced using a microtome and stained with hematoxylin-eosin (H&E) and Masson's trichrome for conventional microscopic examination.

Immediately after extraction, the specimens for ultrastructural study were fixed in 2.5% glutaraldehyde (Fluka, Buchs, Switzerland) for 4 hours at 4°C and demineralized in EDTA. The portion was subsequently fixed with 1% osmium tetroxide for 2 hours, then washed and dehydrated in a series of progressively stronger acetone solutions. Tissue blocks were enclosed in Epon 812 resin (TAAB, Berkshire, England). Semithin toluidine blue-stained sections were evalu-

ated using a high-resolution microscope linked with a digital camera; selected areas were analyzed by electron microscopy. Ultrathin sections were prepared with a diamond knife and a Reichert ultramicrotome (Danaher/Leica Microsystems, Wetzlar, Germany) and contrasted with uranyl acetate and lead citrate. Stained sections were observed at 60 kV with a JEM-1010 transmission electron microscope (JEOL, Tokyo, Japan).

### Platelet Counts and Levels of Growth Factors in PRGF

Forty-three volunteers aged 20 to 72 years (28 women and 15 men; mean age,  $42 \pm 16$  years) allowed samples of their blood to be analyzed. After the subjects gave their informed consent, a sample (10 mL) of whole blood was drawn from each subject. PRGF was then prepared as described. The leukocyte and platelet counts were determined in the whole blood and in the PRGF before clotting (ABX Diagnostics Micros 60, Montpellier, France). Platelet-derived growth factor-AB (PDGF-AB), transforming growth factor-beta1 (TGF- $\beta$ 1), insulin growth factor-I (IGF-I), vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), and epidermal growth factor (EGF) were quantified in the supernatants released from platelet-rich matrices using Quantikine colorimetric sandwich enzyme-linked immunosorbent assay (ELISA) kits (R&D, Minneapolis, MN) according to the manufacturer's instructions and as described previously.<sup>13</sup>

### Case Study

A case study was selected to illustrate the successful use of this technique.

### Statistical Analysis

Values are expressed as means  $\pm$  SDs. Pearson correlations were used to examine the relationship between platelet counts in the PRP used for the platelet-rich matrix and secreted PDGF, TGF- $\beta$ 1, VEGF, HGF, and EGF. A difference of  $P < .05$  was considered statistically significant (Statgraphics Plus, Statpoint, Herndon, VA).

## RESULTS

### Drill Temperature

Drilling at 50 rpm without irrigation did not produce overheating at the tip of the drills; temperatures were  $28.1 \pm 1.9^\circ\text{C}$  ( $n = 10$ ) and  $27.5 \pm 0.9^\circ\text{C}$  ( $n = 10$ ) for the pilot and twist drills, respectively.

### Evaluation of Collected Bone

Light microscopic analysis of the human bone obtained by conventional drilling procedures is shown in Figs 3a and 3b. Although some conserved trabeculae were seen, osteocyte lacunae were empty, and no living cells were found. Conversely, the tissue particles obtained by the alternative new drilling procedure at low speed conserved the bone architecture and included well-preserved trabeculae, where osteocytes, osteoblasts, and lining cells could be found (Figs 3c to 3f). Additional ultrastructural analysis of these tissue sections showed preserved lining (Fig 3g) and osteocytic cells (Fig 3h) that may assist the regenerative process.

Different ratios of cortical to cancellous bone were observed among samples depending on the patient and the intraoral location.

### Platelet Counts and Levels of Growth Factors in PRGF

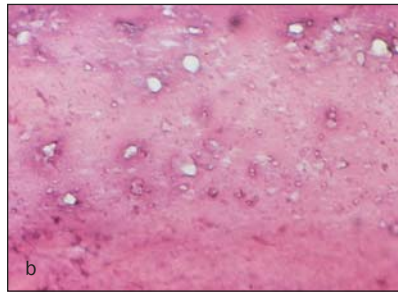
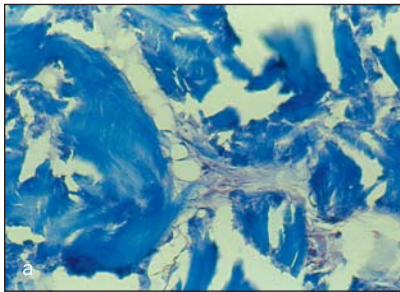
The whole blood baseline platelet count ranged from 153,000 to 359,000 platelets/ $\mu\text{L}$ ; all were within the normal human range. The plasma enriched in platelets increased the platelet number on average from 212,000 (SD 53) to 590,000 (SD 197) platelets/ $\mu\text{L}$ . White blood cells were below the detection limit of the counter. Mean levels of EGF, VEGF, and HGF were  $455.49 \pm 210.04$  pg/mL,  $421.09 \pm 399$  pg/mL, and  $605.70 \pm 269.20$  pg/mL, respectively. Mean IGF-I, PDGF-AB, and TGF- $\beta$ 1 concentrations were significantly higher; they reached  $88.62 \pm 38.36$  ng/mL,  $27.96 \pm 12.13$  ng/mL, and  $55.27 \pm 16.23$  ng/mL, respectively. Moreover, TGF- $\beta$ 1 ( $r = 0.8028$ ), PDGF-AB ( $r = 0.7174$ ), EGF ( $r = 0.5748$ ), VEGF ( $r = 0.3654$ ), and HGF ( $r = 0.4009$ ) showed significant correlation with platelet count ( $P < .05$ ). EGF showed a significant positive correlation with HGF and TGF- $\beta$ 1 ( $P < .05$ ). PDGF showed a significant positive correlation with TGF- $\beta$ 1 and VEGF ( $P < .05$ ).

### Clinical Application: Case Study

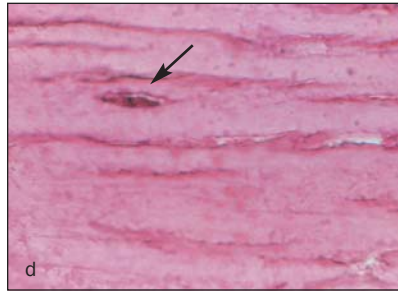
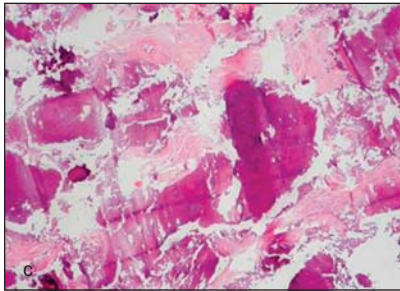
A 46-year-old male patient with a vertical fracture of the maxillary left central incisor presented for treatment. The tooth was extracted, and a 4.5-mm-diameter, 13-mm-long implant (BTI Implant System) was placed. Three screw threads remained exposed. Consequently, the whole area was covered with autologous bone obtained by means of the low-speed drilling procedure mixed with PRGF. The site was subsequently covered with a fibrin membrane obtained as described in the PRGF protocol (Figs 4a to 4c). After 4 months, stability of the implant as measured using an Osstell device (Integration Diagnostics, Göteborg, Sweden), and was found to be 72. The definitive restoration was then fabricated (Figs 4d to 4f).



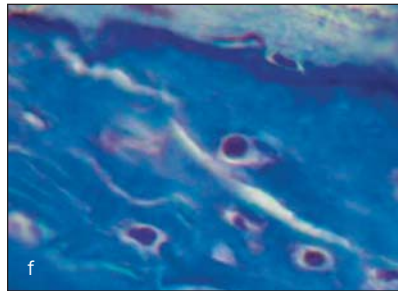
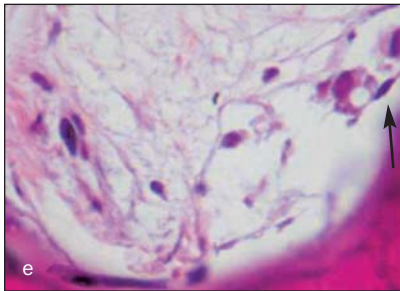
**Fig 3** Representative microphotographs of optic and ultrastructural analyses of both human bone particles obtained during conventional drilling and those obtained during low-speed drilling.



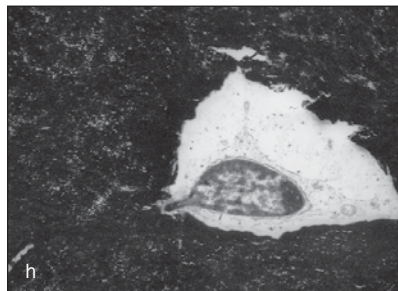
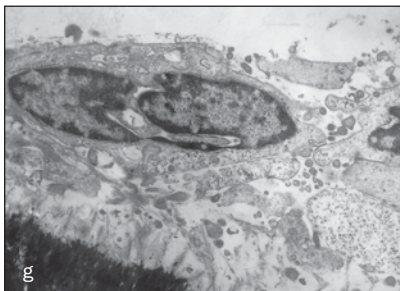
**Figs 3a and 3b** Bone collected after high-speed drilling: (a) trabecular bone (Mason trichrome; original magnification  $\times 10$ ) and (b) compact bone (H&E; original magnification  $\times 10$ ).



**Figs 3c and 3d** (c) Optic microscopic image of human trabecular and compact bone collected after low-speed drilling (H&E; original magnification  $\times 10$ ). (d) One osteocyte (arrow) can be observed surrounded by mineralized matrix (H&E; original magnification  $\times 60$ ).



**Figs 3e and 3f** (e) Representative optic microscopic image of human bone collected after low-speed drilling showing surface cells (arrow) that may correspond with lining and osteoprogenitor cells (H&E; original magnification  $\times 60$ ). (f) Osteocytes inside the bone matrix (Mason trichrome; original magnification  $\times 60$ ).



**Figs 3g and 3h** Ultrastructural study of bone particles obtained during low-speed drilling. Electron micrographs illustrating (g) well-preserved human bone cells for either cell surface (lining and osteoprogenitor cells) and (h) osteocyte cell found in bone lacuna (original magnification  $\times 5,000$  for both images).

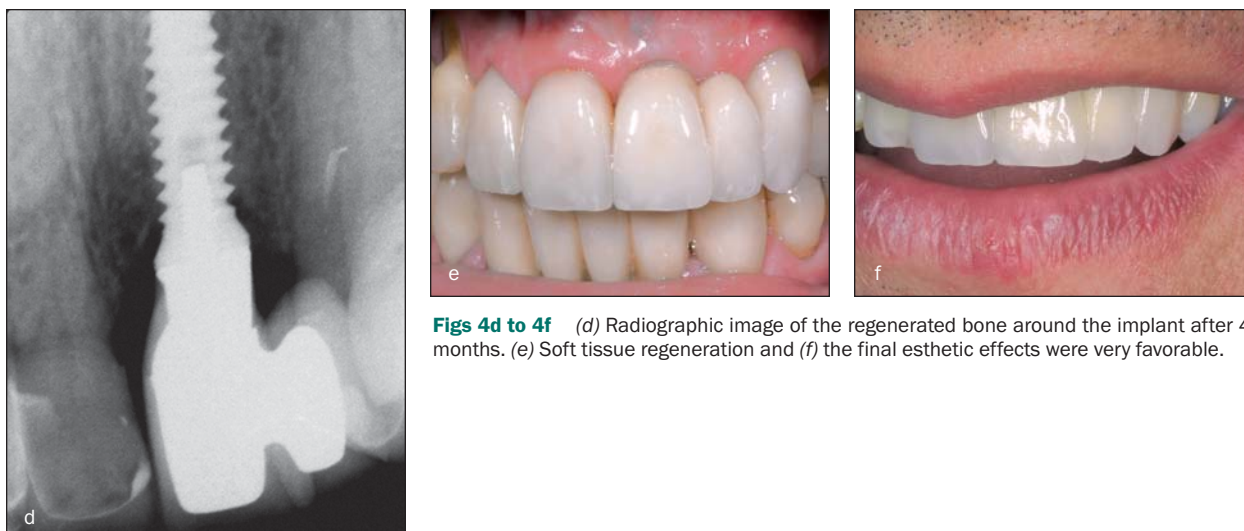
## DISCUSSION

The concept of drilling presented here has been suggested as an alternative to the conventional procedure. It provides a method for obtaining autologous bone during preparation of the surgical site, eliminating the need to collect bone from a second surgical area. Once the cortical layer has been drilled with

a very sharp drill, the bone tissue becomes less dense and contains more cells. Therefore, using the described procedure, low-speed, nonirrigation drilling is conceivable. The fact that the drill is used at low speeds helps improve the quality of tissue obtained, as evidenced by light microscopic and ultrastructural analysis (Fig 3) and the biologic conditions of bone tissue in the neo-alveolus.



**Figs 4a to 4c** (a) As a consequence of the osseous fracture, 3 screw threads remained exposed. The regenerating area was managed as follows: (b) The bone defect was filled with autologous bone mixed with PRGF, and (c) the whole area was covered with a fibrin membrane obtained as described in the PRGF protocol.



**Figs 4d to 4f** (d) Radiographic image of the regenerated bone around the implant after 4 months. (e) Soft tissue regeneration and (f) the final esthetic effects were very favorable.

One widespread practice used in conventional drilling techniques to avoid thermal damage is to apply apyrogen water or saline irrigation to the drill bit to prevent the bit and the surrounding tissue from overheating. The effects of drill speed on heat production have been studied to improve irrigation delivery systems.<sup>14,15</sup> However, irrigation washes away signaling proteins and other soluble substances that play an active role in bone regeneration. Flood irrigation, either with apyrogen water or saline, can drag and dissolve osteoinductive signaling proteins present in the bone extracellular matrix, such as bone morphogenetic proteins, growth factors, and those synthesized in response to the drill insult.<sup>16</sup> The specific physiologic function of these signaling proteins is to transmit activation messages to the local cells so that they can react to the deterioration suffered in the microenvironment.<sup>17,18</sup> Several of these proteins are bonded to the extracellular matrix, and this connection is broken when the drill comes into contact with the matrix. Inasmuch as these signaling proteins are characterized by their low molecular weight and solubility, saline irrigation easily dissolves and washes them away, thereby stripping the tissue of the natural resources it uses to heal itself.<sup>19</sup>

Although the biologic properties of the host tissue after drilling cannot be measured directly by any method, analysis of displaced particles has shown that they contain living bone; it may be possible to extrapolate this experimental evidence to the neo-alveolus. Moreover, the fact that drilling is performed at low speed helps improve the quality of tissue obtained, as the particles obtained with this method are larger. As the drill completes considerably more rotations at high speed than at low speed, yet advances the same distance, high-speed drilling shreds the tissue to a greater extent.

Several authors<sup>20,21</sup> have compared bone particles obtained from different commercial bone traps and examined differences in the percentage of bone to coagulum. Bone trap design also affects the mass and the nature of the collected tissue.<sup>22</sup> The presence of microorganisms found during the collection process can be very high due to saliva retention with some bone traps.<sup>20</sup> However, the displaced tissue retained in the drill bit during low-speed drilling is very easy to collect, lessening saliva contamination.

The protocol described here used PRGF for the local application of bone graft material. Immediately

after activation, PRGF is mixed with the bone graft, and the platelet-rich fibrin matrix that develops subsequently confines the bone particles together, making its application and adaptation to the injured site easy. The numerous proteins secreted by the activated platelets may influence aspects of healing such as chemotaxis and mitogenesis of stem cells and osteoblasts, angiogenesis for capillary ingrowth, bone matrix formation, and collagen synthesis. Once PRGF is activated, a large list of growth factors and proteins potentially of great value in bone healing and graft remodeling are released from the platelet-rich fibrin matrix, including TGF- $\beta$ 1, PDGF, IGF, bone sialoprotein, thrombospondin, osteocalcin, and osteonectin.<sup>5</sup>

Furthermore, some delivered factors promote neovascularization both in vivo and in vitro.<sup>23</sup> Although the angiogenic activity appears to be predominantly due to VEGF and HGF, other growth factors, such as TGF- $\beta$ 1, are involved in their regulation.<sup>24</sup> Blood vessel invasion of the graft is essential if proper incorporation and remodeling are assumed to achieve optimal bone repair. Several authors have described the efficacy of PRPs as bone graft enhancers when used in combination with autogenous bone, lyophilized bone, or bone substitutes<sup>25</sup>; other conflicting and inconclusive results regarding the potential benefits of this procedure prompt the need for suitable definitions and characterization for the different PRP preparations currently being tested.<sup>26</sup> Further knowledge of growth factor networks and identification of their activities in the context of PRPs is needed to ascertain the optimal amounts of platelets and/or the most appropriate balance between the different elements. Given the number of variables and their potential for interaction, it seems difficult to establish a single recommendation of platelet number based on scientific criteria. Furthermore, several additional factors, such as the type of clot activator, the platelet arrangement within the fibrin scaffold, the leukocyte content, and the time that the fibrin scaffold is put into place after clotting has started can markedly influence the different biologic effects. Therefore the nature of each PRP and the protocol for application should be precisely described. PRGF contains a moderately elevated platelet concentration of approximately  $600 \times 10^3$  platelets/mL and is activated by the addition of calcium chloride. Activation mimics physiologic clotting, allowing a more sustained release of growth factors that might be crucial in the proper tissue repair and wound healing.<sup>27</sup> Moreover, it obviates immunologic reactions and the risk of disease transmission associated with the use of exogenous thrombin.<sup>28</sup> Eventually the presence of leukocytes in

these preparations may be of great significance, since they contribute to the heterogeneity of the products, providing a pool of additional cytokines and interleukins. PRGF does not contain neutrophils or other leukocytes; thus, it may exhibit improved homogeneity and less donor-to-donor variability compared with other platelet-rich collection systems,<sup>29</sup> making it a safer and more effective product.

The absence of neutrophils is especially relevant, as neutrophils express matrix-degrading enzymes, such as matrix metalloproteinase-8 (MMP-8) and MMP-9, and release reactive oxygen species that destroy surrounding cells.<sup>30</sup>

The use of a composite graft made of autogenous bone obtained from the surgical preparation site and PRGF may be very advantageous in grafting procedures intended to achieve optimal bone regeneration. However, identification and prioritization of critical factors for bone regeneration need to be addressed to establish the optimal graft.

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## Book Review

*Practical Periodontal Plastic Surgery* by Serge Dibart and Mamdouh Karima. Ames, IA: Blackwell Munksgaard, 2006.

This nicely illustrated textbook provides historical background information on the field of periodontal plastic surgery and covers the progress made in all aspects of periodontal plastic surgery to date. The majority of the book covers common periodontal plastic surgeries; however, pertinent periodontal plastic procedures related to implant therapy are also nicely illustrated and discussed. For example, the history of and indications and technique for the subepithelial connective tissue graft are presented for natural teeth and implants. Additional implant-related topics include preprosthetic hard and soft tissue augmentation procedures. In the section on soft tissue augmentation procedures, the connective tissue graft is the primary procedure discussed, while hard tissue augmentation procedures presented include autografts, allografts, xenografts, and synthetic grafts. Additional implant-related topics include socket preservation, ridge splitting, and ridge-expansion techniques.

An entire chapter is devoted to soft tissue management around dental implants. This chapter discusses the biologic width, gingival biotypes, and soft tissue grafting around implants for both esthetic procedures and function. Soft tissue stage-2 procedures are nicely illustrated; examples of connective tissue grafts, free gingival grafts, an apically positioned flap, and a buccally positioned envelope flap are presented.

The majority of this text covers periodontal soft tissue grafting procedures; however, adequate coverage of soft and hard tissue management of implant therapy is also presented with excellent illustrations. This text is recommended as a valuable addition to the libraries of both graduate students in periodontics and prosthodontics and recent graduates.

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