Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part V: Histologic evaluations of PRF effects on bone allograft maturation in sinus lift

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Objective. Platelet-rich fibrin (PRF) belongs to a new generation of platelet concentrates, with simplified processing and without biochemical blood handling. The use of platelet gel to improve bone regeneration is a recent technique in implantology. However, the biologic properties and real effects of such products remain controversial. In this article, we therefore attempt to evaluate the potential of PRF in combination with freeze-dried bone allograft (FDBA) (Phœnix; TBF, France) to enhance bone regeneration in sinus floor elevation.

Study design. Nine sinus floor augmentations were performed. In 6 sites, PRF was added to FDBA particles (test group), and in 3 sites FDBA without PRF was used (control group). Four months later for the test group and 8 months later for the control group, bone specimens were harvested from the augmented region during the implant insertion procedure. These specimens were treated for histologic analysis.

Results. Histologic evaluations reveal the presence of residual bone surrounded by newly formed bone and connective tissue. After 4 months of healing time, histologic maturation of the test group appears to be identical to that of the control group after a period of 8 months. Moreover, the quantities of newly formed bone were equivalent between the 2 protocols.

Conclusions. Sinus floor augmentation with FDBA and PRF leads to a reduction of healing time prior to implant placement. From a histologic point of view, this healing time could be reduced to 4 months, but large-scale studies are still necessary to validate these first results.

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Elevation of the sinus floor to increase the alveolar bone needed to place implants is considered to be a highly predictable and effective treatment option.[1-3] Many techniques have been described to achieve vertical augmentation of the maxillary sinus mucosa. When considering a lateral approach to the sinus, the major differences between the various surgeries consist of the type of grafting material used and the decision of immediate or delayed implant placement.[4] In case of severe atrophy of the maxillary alveolar process, sinus floor elevation and implant insertion are usually performed in 2 stages.[5] When autogenous bone graft is used, it takes approximately 6 months following augmentation for the transplanted bone to be integrated and substituted by osteoconduction (creeping substitution). Alternatively, autogenous bone transplants can be replaced by bone substitutes, eg, freeze-dried bone allograft (FDBA), to avoid donor site morbidity.[6] Maturation of these materials may take up to 8 months if used for sinus augmentation.

It would be beneficial for the patient to reduce this time interval by accelerating the process of the transplanted bone or the bone substitute. Use of platelet-rich plasma was a promising option that remains controversial.[7-25]

Use of fibrin glue to improve bone regeneration is well documented.[26-32] (Platelet-rich fibrin (PRF) is an autologous fibrin matrix used to enhance bone

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Table I. Healing time according to the graft material used

Graft material	*Healing time*	*Number of cases*
FDBA	8 months	3
FDBA	4 months	Impossible sample
FDBA+PRF	4 months	6

FDBA, freeze-dried bone allograft; *PRF,* platelet-rich fibrin.

generation.[33,34] The aim of this histologic study is to evaluate the potential of PRF in combination with FDBA to enhance bone regeneration in sinus floor elevation.

MATERIALS AND METHODS

Patient selection

This study is a case of 9 sinus elevations performed between January 2001 and June 2003 with FDBA (Phoenix; TBF, France) with or without PRF (Table I). The study was conducted in accordance with the standards of the Declaration of Helsinki of 1983. The patients were informed about the aim and design of the study and written consent was obtained.

Patients with immunologic diseases, unstable diabetes mellitus, ongoing chemo- or radiotherapy, or a history of drug abuse were excluded. The inclusion criteria were a blood concentration of thrombocytes within the normal range and an absence of a history of maxillary sinus inflammations. Clinical examination and preoperative radiographs showed a severe atrophy of the maxilla.

Surgical procedure

Surgery was performed with local anesthesia. Access to the lateral maxillary wall was achieved via a mucosal crestal incision, and anterior and posterior releasing vestibular incisions. A bony window of approximately 15-20 mm^2 was outlined by a round bur with constant saline irrigation. It was then moved medially and left in that position, still attached to the sinus membrane. After careful elevation of the schneiderian membrane without perforation, 1-2 g of Phoenix containing FDBA granules of 200 to 800 μm diameter were instilled for augmentation of the sinus floor. In 3 cases, the sinus was filled with FDBA only (control group). In the 6 other cases, PRF was added to the bone graft particles (test group).

PRF preparation

The PRF was produced using the technique previously described.[33] The patient's blood samples were taken during the surgery in the operating room, prior to the sinus elevation. Immediately after the blood draw, the dried monovettes (without anticoagulant) were centrifugated at 2,500 rpm (about 280*g*) for 10 minutes in a laboratory centrifuge (Process, Nice, France). The PRF clots were recovered and used in 2 ways:

— Some were placed in sterile cups and cut in few-millimeter fragments. Then they were mixed with FDBA particles. The mixture obtained constituted an easy-to-use homogeneous graft material.
— Others were packed tightly in 2 sterile compresses in order to obtain resistant fibrin membranes transferable to the schneiderian membrane (to prevent or treat perforation) and on the grafting material before wound closure. They can also be placed under the incision line to improve mucosal healing.

Harvesting of the bone specimen

Implant insertion was performed 4 months following sinus floor augmentation for the test group and 8 months for the control group. During this procedure, a bone biopsy from the augmented site was harvested using a trephine bur of 3 mm diameter. To guarantee that the augmented region of interest was examined, a drill was used before the trephine bur to eliminate the superficial and nonregenerated bone.

The healing time and number of collected bone specimens are summarized in Table I. In the control group, bone sampling after a healing time of 4 months was not possible. That is why this is left without comment in the present study.

Histologic examination

Bone fragments were removed, fixed in formaldehyde solution, dehydrated in alcohol, and embedded in methylmethacrylate resin. Undecalcified sections were made and stained according to 2 protocols: toluidine blue/PAS and Masson trichrome staining. The images were assessed at magnification 100× to 630× for qualitative analysis and digitized for quantitative analysis. From digital images of these sections, different histologic structures were separated and measured (in pixels) using image analyzer software.

In staining by Masson trichrome, mineralized trabecular bone is identified in green, osteoïd borders in red, and medullary spaces in pink (Fig. 1, *A* and *B*). In staining by toluidine blue/PAS, mineralized matrix appears blue, osteoïd borders are red, and medullary spaces are orange-pink (Fig. 1, *C* and *D*). Measures of each histologic structure are expressed in total section area percentage (Figs. 2 and 3).

It is difficult to differentiate new bone formation and FDBA particles, because both collagenous matrixes are very similar. Therefore, a meticulous histologic observation of bone vitality is necessary to quantify the new bone areas: When osteocytic lacunas are filled with a well distinguished osteocyt, it is new vital bone. On the other hand, when the lacunae are empty, it is inert graft bone. Thus, histomorphometric evaluation is dependent on the operator. That is why in this study,

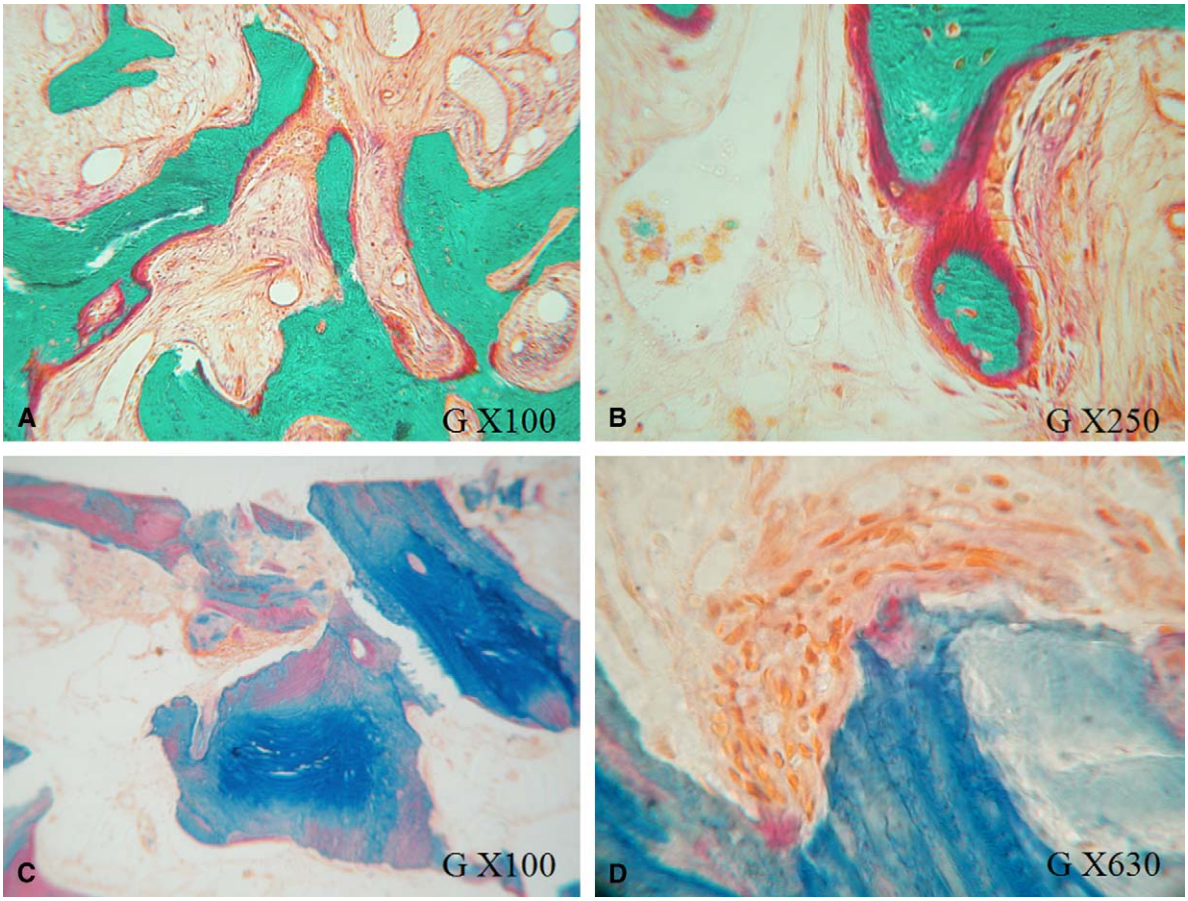

Fig. 1. Preliminary analyses highlight mineralized trabecular bone rich in osteocytes which appear green (**A** and **B**) or blue (**C** and **D**) according to the staining. Osteoïd borders are stained in red (**B** and **D**) and are in contact with dense cellular osteoblast fronts. The richness of osteoïd tissue is evidence of important turnover in both types of samples (test and control).

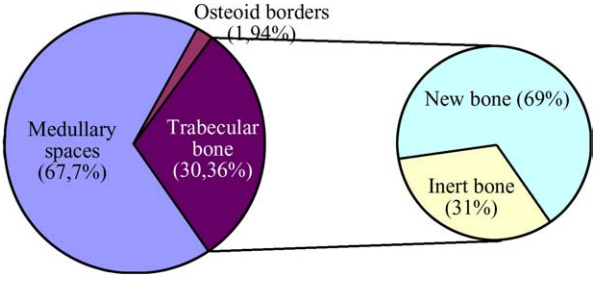

Fig. 2. Mean histomorphometric analysis of bone samples from 3 sinus floor augmentations after a healing period of 8 months (control group: FDBA alone).

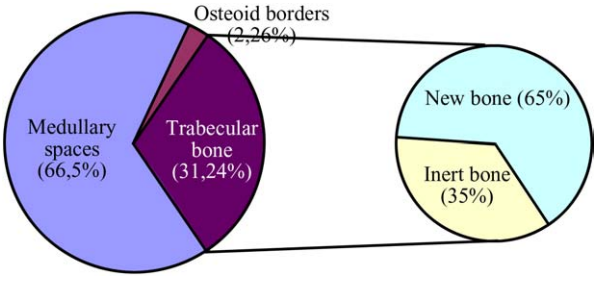

Fig. 3. Mean histomorphometric analysis of bone samples from 6 sinus floor augmentation after a healing period of 4 months (test group: FDBA+PRF).

histomorphometric evaluations were performed by 3 different laboratories.

RESULTS

Preliminary analyses highlight mineralized trabecular bone rich in osteocytes with important oteoïd borders in contact with dense cellular osteoblast fronts (Fig. 1). Nevertheless, trabecular bone areas are less massive, more spaced and surrounded by adipose tissue. These observations especially concern apical parts of the samples. This phenomenon is explainable by the difficulty of correctly packing the bone graft particles in the entire sinus cavity during surgery. Even if such areas are less dense, they represent strong matrix turnover activity.

Table II. Statistical results of histomorphometric quantification within bone allograft samples, with or without PRF

Bone graft	*Clinical reports*	*Bone type*	*Range*	*Average*
FDBA only After 8 months	3	Non vital bone.	9.28% - 12.206%	10.934%
		Vital bone.	18.02% - 23.694%	20.306%
FDBA and PRF After 4 months	6	Non vital bone.	9.03% - 12.7%	9.41%
		Vital bone.	18.65% - 30.3%	20.95%

FDBA, freeze-dried bone allograft; *PRF,* platelet-rich fibrin.

In one case, a perforation of the sinus membrane was treated using the PRF membrane. After this fibrin membrane placement, the sinus filling was able to be completed. Four months later, histologic evaluation showed normal bone density.

The rate of vital bone/inert bone in the bone trabecular areas makes it possible to evaluate the importance of turnover. One can observe one-third inert bone graft and two-thirds new vital bone (Figs. 2-3, Table II) for both groups (FDBA and FDBA+PRF). The importance of osteoïd tissue in both types of sample gives evidence of substantial turnover. Finally, the histomorphometric results of control group (FDBA without PRF) after 8 months appear equivalent to those of the test group (FDBA with PRF) after 4 months. This fact constitutes the essential strength of these histologic observations. It is the first evaluation of the quality of new bone formation within the bone graft when PRF is added to FDBA in case of sinus lift augmentation after 4 months healing time.

DISCUSSION

The aim of this histologic study was to evaluate the potential of PRF in combination with freeze-dried bone allograft to enhance bone regeneration in sinus floor elevation. Histomorphometric analysis shows that bone structures between control group (FDBA alone) and test group (FDBA+PRF) seem to be similar. But the healing period of the 2 groups was not identical (8 months and 4 months, respectively). Therefore, use of PRF with FDBA to perform sinus floor augmentation seems to accelerate bone regeneration and allow implant placement after only 4 months of healing. Thus, healing time between sinus graft and implant placement could be considerably reduced by using PRF.

These histologic analyses highlight other advantages of using PRF. PRF adjunction to FDBA makes it possible to enhance the graft volume without injuring the maturation quality in new bone. That is why, in the case of autogenous graft, addition of PRF has to be tested to show if it can lead to a reduction of the volume of bone harvesting.

From a fundamental point of view, it is still difficult to know if the addition of a fibrin clot really permits enhancement of new bone deposit. Nevertheless these histologic results concur with other studies focusing on the rule of fibrin network on tissue regeneration.[35-37] This fibrin matrix will guide the healing processes. PRF contains platelet growth factors as well, but these cytokines seem to have a secondary rule in the bioactivity of PRF. This hypothesis can be reinforced by the histologic evaluation of the osteocyt number in both control and test group samples, which is identical. Therefore, PRF does not appear to enhance cellular proliferation in the long term, but may play an important role in the revascularization of the graft by supporting angiogenesis.

CONCLUSIONS

In this study, the histologic similarities observed between these 2 groups (FDBA alone and FDBA+PRF) make it possible to consider sinus floor augmentation surgery with a shorter healing period before implant placement (4 months instead of 8 months). Furthermore, the quantity of bone material used to fill the sinus cavity can be safely reduced without injuring the final bone density. Finally, the PRF membranes appear to be able to treat sinus membrane perforation and permit the surgery to be completed. The use of PRF, in addition to a bone graft material, to perform sinus floor augmentation is attractive from a histologic point of view. Nevertheless, other major prospective clinical studies must be conducted to validate the healing period of 4 months between sinus floor procedures and implant placement.

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