Human Recombinant Tissue Factor, Platelet-rich Plasma, and Tetracycline Induce a High-Quality Human Bone Graft: A 5-year Survey

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Purpose: *To increase human bone graft regeneration and quality by the use of a mixture containing autologous ground calvarial bone, human recombinant tissue factor (rhTF), platelet-rich plasma (PRP), and tetracycline.* ***Materials and Methods:*** *Maxillary sinus floor augmentation was performed on 18 patients by grafting a "bone paste" made of PRP (1.8×10^6 platelets/mm^3 plasma), about 1 µg rhTF, calvarial bone chips (2 to 5 mm in size), and tetracycline (10 to 30 µg/mL preparation). Five to 6 months after the surgical phase and grafting, a bone core was extracted for implant fixation, and the osseous core samples were analyzed microscopically.* ***Results:*** *Histology revealed vascularized connective tissue rich in lamellar bone spicules containing osteocytes and surrounded by osteoblasts. The success rate of grafting was 90.3%. In 6-month postoperative blood samples, no residual coagulating disturbances could be found.* ***Discussion:*** *The combination of calvarial bone chips, rhTF, PRP, and tetracycline results in a paste that is easy to handle, safe for patients, and possesses high bone-regeneration capacity.* ***Conclusion:*** *The generalized use in implant dentistry, oral surgery, and orthopedics of such a protocol could facilitate the healing process as well as patient safety and surgeon comfort.* (Int J Oral Maxillofac Implants 2003;18:411–416)

Key words: *autologous bone graft, dental implants, histology, platelet-rich plasma, sinus augmentation, tissue factor*

Bone grafts are widely used in craniomaxillofacial surgery to increase skeletal jaw volume, bridge bony defects, and stabilize bone segments. Several investigations have attempted to explore various aspects of autogenous and allogeneic bone graft incorporation to optimize graft survival and maintenance of graft volume.[1–6] The use of a high concentration of platelets containing growth factors can modulate and enhance wound healing and tissue and bone regeneration.[7–11] In this report the successful use of a combination of recombinant human tissue factor (TF, or tissue thromboplastin), a solution of autologous living platelet-rich plasma (PRP) mixed with autologous calvarial particulate bone graft containing minocycline,[12,13] will be presented. The association of these 4 items in paste form can be useful for reconstructive surgery, preprosthetic surgery, and reconstruction of ridge atrophy (Fig 1).

In vivo, the major pathway of blood activation is the extrinsic pathway, which includes TF (or FIII) and plasma factor VII (FVII). TF is a distant member of the cytokine hematopoietic growth factor receptor superfamily. TF functions as a cofactor/receptor, which in the presence of calcium activates FVII. It results primarily from binding of FVII to TF/Ca^{2+}. This complex acts on 2 substrates, FX and FIX, to generate thrombin.[14–19] TF is considered to be the

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Fig 1 Paste generated after mixture of autologous bone chips, PRP, rhTF, and tetracycline.

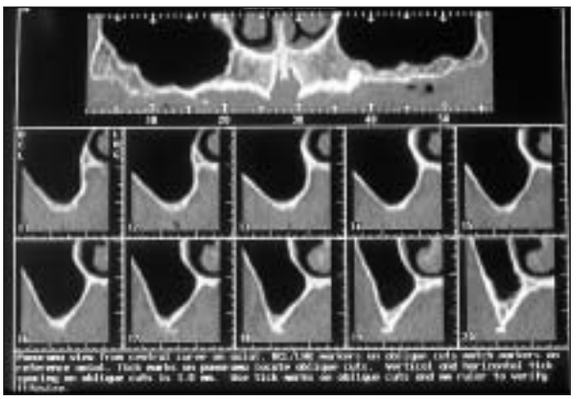


Fig 2a *(Right)* Maxillary CT scan of edentulous maxilla before surgical reconstruction, presenting complete bilateral maxillary pneumatization (class VI according to Cawood and Howell[29]).

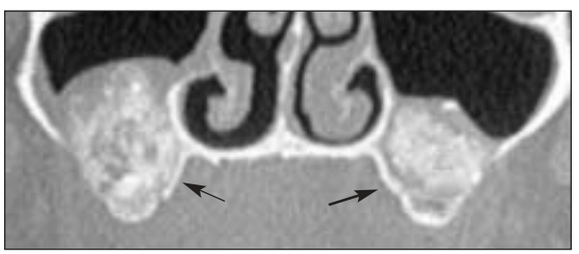


Fig 2b CT scan taken 2 months after surgical filling procedure. Arrows illustrate the homogeneous filling of the whole cavity.

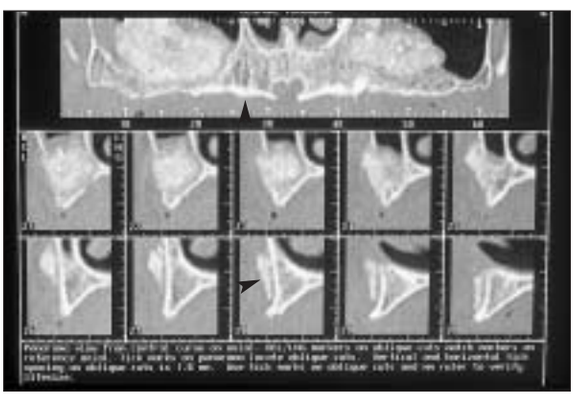


Fig 2c *(Right)* On section 28 *(arrow)* paste apposition can be seen on the premaxillary area.

physiologic trigger of the blood clotting system in normal hemostasis.[17,20] More recently, there has been a growing interest in this protein, a new member of the cytokine receptor superfamily, acting as a signaling receptor,[21] and it has been suggested that TF may have additional important non-hemostatic roles.

The complex TF + FVIIa acting on human fibroblasts leads to activation of phospholipase C and enhanced platelet-derived growth factor-BB–stimulated chemotaxis,[22] induces proinflammatory effects in macrophages,[23] and increases vascular endothelial growth factor production by human fibroblasts.[24]

MATERIALS AND METHODS

Since 1997, 18 patients (17 women and 1 man, mean age 52 years, range 40 to 64) with severe atrophy of the maxillary alveolar process, diagnosed by panoramic radiographs and by computed tomography (CT) (Fig 2a), have been included in this study.

Nine patients were edentulous, and 7 had a unilateral or bilateral loss of molars or premolars. Two had lost teeth in the premaxilla. A total of 25 maxillary sinuses that had less than 4 mm (range 1 to 3 mm) of subantral alveolar bone in the vertical direction were treated with an augmentation procedure by filling with bone paste and delayed implant placement. The regional ethical research committee approved the study (Association of Public Hospitals "Iris South," Brussels).

Calvarial Bone Graft Harvesting

Grafted bone can be obtained from trabecular, cortical, or corticotrabecular sources. Trabecular grafts provide osteogenic cells; cortical grafts have fewer surviving osteogenic cells but provide more bone morphogenetic protein (BMP).

Harvesting of medullocorticoparietal skull grafts was performed under general anesthesia and without antibiotic prophylaxis. A unicortical osteotomy was performed using a thin fissure bur. The same protocol can be used under local anesthesia with cortical mandibular bone harvesting when less bone paste is needed. The harvesting site was divided in thin strips and removed piece by piece.[25] The bone was kept in isotonic solution (NaCl 0.9%) containing 50 µg/mL minocycline (Haupt Pharma Cyanamid, Wolfratshausen, Germany). The periosteum and muscle were sutured in one layer and the scalp in a second layer, and then a compressive bandage was placed.

Maxillary Sinus Augmentation Technique

A crestal incision was performed with vertical releasing incisions, and then a mucoperiosteal flap was elevated and reflected laterally to expose a thin bone plate, which was easily penetrated with rotating instruments. The fragile Schneiderian membrane did not play an important role for the containment of the bone graft, because the sinus was filled with the paste. During the elevation, the membrane was often perforated (range of 33%).

Gelification of Bone Chips

The corticotrabecular graft was ground in a bone mill (Friadent, Mannheim, Germany) to obtain approximately 5 mL of bone chips. Two milliliters of a buffered solution (sodium bicarbonate [$NaHCO_3$]) 0.6 mol/L, pH 7.4) containing 100 µg/mL minocycline (Haupt Pharma Cyanamid) was added, followed by 5 mL of PRP (10^6 platelets/mL) (Red Cross, Brussels, Belgium). This sterile 12-mL "granulated solution" was maintained at 37°C in a water bath. Recombinant human (rh) TF (innovin lyophilized thromboplastin, Dade Behring International, Paris, France) was dissolved in 2 mL of sterile water containing 1 µg of rhTF and then added to the granulated solution. Fibrin clot was apparent in less than 1 minute.[26–28] The paste was placed on sterile gauze and cut into several pieces to be applied according to the need for either a sinus lift procedure in the maxilla[29] (inlay bone graft) (Fig 2b) or graft apposition (onlay bone graft) (Fig 2c) in the canine region. The gel was easy to handle and did not easily migrate or penetrate holes in the membrane. The wounds were sutured with silk sutures.

Biopsy Procedure and Implant Placement

Bone core harvesting and implant placement were carried out after a healing period of 4 to 6 months. In 18 patients, core samples were taken at the time of implant placement, in an axial direction from the residual alveolar bone into the grafted area with a trephine (5-mm diameter, Nobel Biocare, Göteborg, Sweden). Implants (diameter 5.5 mm; Frialit-2, Friadent) were systematically placed in the site where the bone core was extracted. The average length of the bone core was 14 mm (range, 12 to 17 mm) (Fig 3).

To be considered successful, a patient had to have sufficient bone regenerated to place implants that were at least 13 mm long. All patients were seen at least 3 times per year, and the following evaluations were made upon removal of the prostheses: implant immobility, clinical attachment level, tissue margin height, and Gingival Index. No attempts were made to accomplish progressive loading of the newly regenerated bone, other than what occurred during the time in which the restorative dentist proceeded from temporary to definitive restorations (median range: 8 weeks).

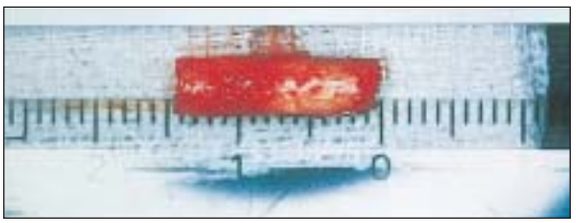


Fig 3 Macroscopic view of an extracted bone core (scale in centimeters). Note the visible osseous gradient of this 15-mm core; 0 mm corresponds to the buccal region.

Histologic Preparation and Assessment

All bone samples were immediately immersed in a buffered 4% paraformaldehyde fixative for 24 hours and further decalcified for 10 days in a solution containing 5% (v/v) formic acid, 7% (w/v) amonium chloride (NH_4Cl), and 1.4% (v/v) hydrochloric acid. The decalcified bone core was dehydrated and embedded with paraffin; 6-µm sections were cut and stained with hematoxylin-eosin for microscopic analysis.

RESULTS

Bone Scintigraphy

Technetium-99m-methylenediphosphate (Tc-99-MDP) was used to obtain information concerning bone growth and osteoblast activity and can be quantified or qualitatively examined using a gamma counter.[30–33] All examined patients showed a heavy concentration of radioisotopes on the graft side. The radioisotopic images of the skull (Fig 4) show within the grafted region an intense concentration after 4 months. In all patients, the maximum was reached after 6 months and slowly decreased to a normal level after more than 2 years (not shown).

Implant Follow-up

In 22 of 25 maxillary sinuses grafted (3 sinuses with focal infection were excluded), implants (Frialit-2, synchro, stepped screw, deep-profile surface) were placed 5 to 7 months after grafting, depending on the radiographic and scintigraphic analysis. Implants were then allowed to osseointegrate for 6 months before they were uncovered. A total of 58 implants of varying lengths (13 and 15 mm) with widths of 3.8, 4.5, and 5.5 mm, were placed in the grafted subantral area.

Tables 1 and 2 provide information on the success of implants placed into function.

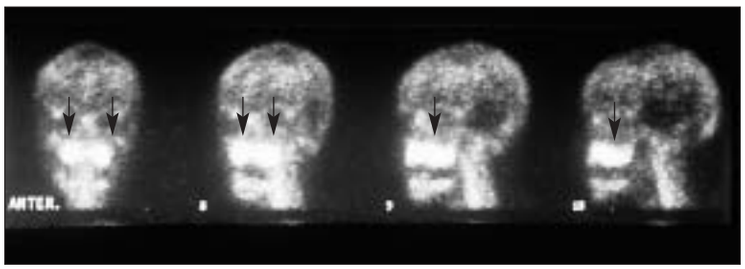


Fig 4 Skull scintigraphy (Tc99-MDP) performed 6 months after surgery. Concentrations within the maxillary grafted region are clearly distinguishable *(arrows).*

Table 1 Rate of Success of Implants Placed in Function

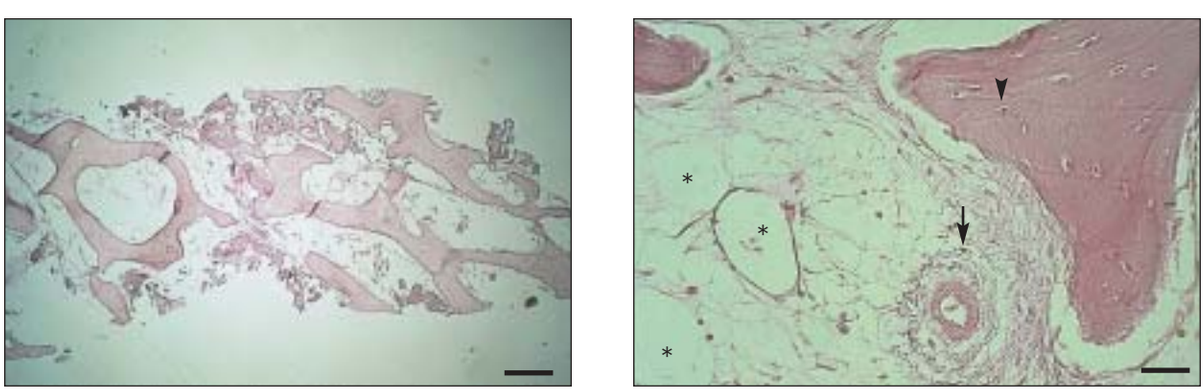
Implant width (mm)	No. placed	No. successful	Percent success
3.8	36	34	94.4
4.5	12	11	91.6
5.5	10	8	80.0*
Total	58	53	91.3

*Implants (cone-shaped) were placed in the cylindric hole of the bone core biopsy.

Table 2 Implant Function over Time

Months in function	No. of patients	No. of implants*
48	1	4
37 to 48	5	25
25 to 36	10	37
13 to 24	15	46
0 to 12	19	53

*Does not include the 5 failed implants.



Figs 5a and 5b Microphotography of the biopsy obtained after 6 months of grafting. Sections (6 µm) were stained with hematoxylin-eosin. *(Left)* Low magnification (bar = 500 µm) gives a view of the reconstructed bone architecture. *(Right)* At higher magnification (bar = 57 µm), the living bone is filled with osteocytes *(arrowhead),* and within the surrounding connective tissue, vascularization is conspicuous *(asterisks)* with few inflammatory cells *(arrow).*

Microscopic Analysis

Histology of the new bone (4 to 6 months after grafting) indicated a well-reconstructed bone with living osteocytes and osteoblasts (Figs 5a and 5b). The connective tissue was highly vascularized, and inflammatory cells were infrequent.

DISCUSSION

The present histologic study suggests that high-quality human bone regeneration occurred following placement of a paste made of PRP gel, rhTF, tetracycline, and autologous material. It can be hypothesized that the intrinsic quality of the gel resulted from the platelet concentrate, a rich source of multiple growth factors,[34,35] combined with rhTF, which produced, through a physiologic pathway, a clot. The beneficial effect of the addition of PRP has previously been shown.[7–11] It is also noteworthy that the added protein was obtained by genetic engineering, which avoids the usual concerns about adverse reactions precipitated by exogenous material (disease transmission and immunogenic reactions). Moreover, it has been shown that the addition of tetracycline to a bone graft may enhance its osteogenic

potential[36] by inhibiting matrix metalloproteinase I (MMP-I) and therefore bone resorption.[37] Thus, the choice and dosage of these ingredients can contribute beneficially to a gel formation that has a high potential for bone regeneration. More data should be accumulated to clearly establish the contribution of each component of the mixture so as to eventually eliminate the less valuable components and propose a more useful and generalized protocol.

CONCLUSION

Hemostasis involves the interplay of 2 biochemical pathways, which are controlled by various proteins, factors, and formed elements, eg, platelets. The process by which blood coagulates, as it is presently understood, involves a multiple-step cascade of activation of the protein factors, which culminates in fibrin formation. The physiologic coagulation pathway (extrinsic and intrinsic) using all factors of the coagulation cascade can be highly amplified by the addition of PRP and rhTF in concentration, leading to the protein scaffold of a blood clot. Numerous studies have shown the value of stimulating an autologous bone graft with PRP. Platelets additionally are known to be the first cellular elements to stimulate regeneration through stimulation of growth factors. However, in vitro, the necessity to stimulate the release of growth factors by platelets within the osseous recipient site remains a challenge. In this work, it has been shown that the association of rhTF with a solution of PRP allows the creation of physiologic, biomimetic habitats for cells needed for bone regeneration and growth. The present protocol appears to fulfill all these conditions and was demonstrated through bone scintigraphy and microscopic analysis.

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