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Platelet-rich plasma

Growth factor enhancement for bone grafts

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Platelet-rich plasma is an autologous source of platelet-derived growth factor and transforming growth factor beta that is obtained by sequestering and concentrating platelets by gradient density centrifugation. This technique produced a concentration of human platelets of 338% and identified platelet-derived growth factor and transforming growth factor beta within them. Monoclonal antibody assessment of cancellous cellular marrow grafts demonstrated cells that were capable of responding to the growth factors by bearing cell membrane receptors. The additional amounts of these growth factors obtained by adding platelet-rich plasma to grafts evidenced a radiographic maturation rate 1.62 to 2.16 times that of grafts without platelet-rich plasma. As assessed by histomorphometry, there was also a greater bone density in grafts in which platelet-rich plasma was added (74.0% ± 11%) than in grafts in which platelet-rich plasma was not added (55.1% ± 8%; $p = 0.005$). **(Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1998;85:638-46)**

In 1994 Tayapongsak et al.[1] introduced the novel idea of adding autologous fibrin adhesive (AFA) to cancellous bone during mandibular continuity reconstructions. They identified earlier radiographic bone consolidation in 33 cases; they attributed this to enhanced osteoconduction afforded to the osteocompetent cells in the graft by virtue of the fibrin network developed by AFA. They also reported the remarkable adhesive advantage of binding cancellous marrow particles during graft placement. Tayapongsak et al.[1] produced their AFA in a blood laboratory setting, separating one unit of whole blood into the red blood cell component and the plasma fraction for use over the following 2 to 3 weeks as a cryoprecipitate. This was then thawed over a 24-hour period to yield a final "fibrinogen-rich concentrate" of 10 to 15 ml.

Since the early 1990s we have been exploring the parallel but more specific sequestration and concentration of autologous platelets in plasma (platelet-rich plasma [PRP]) and studying the growth factors contained within platelets in relation to their biologic enhancement of continuity bone grafts to the mandible. The first purpose of this article is to introduce our studies of PRP; we present data documenting that PRP increases platelet concentration when placed into grafts, showing the presence of at least three growth factors (platelet-derived growth factor [PDGF], transforming growth factor beta 1 [TGF-b_1] and transforming growth factor beta 2 [TGF-b_2]), and indicating that cancellous marrow cells have receptors for these growth factors. The second purpose of the article is to explore the potential of PRP to increase the rate of bone formation in a graft and enhance the density of the bone formed at 6 months. The third purpose of the article is to present a model of bone graft bone regeneration illustrating the mechanism by which PRP may enhance bone regeneration both in rate and amount.

MATERIAL AND METHODS

Eighty-eight elective cancellous cellular marrow bone graft reconstructions of mandibular continuity defects 5

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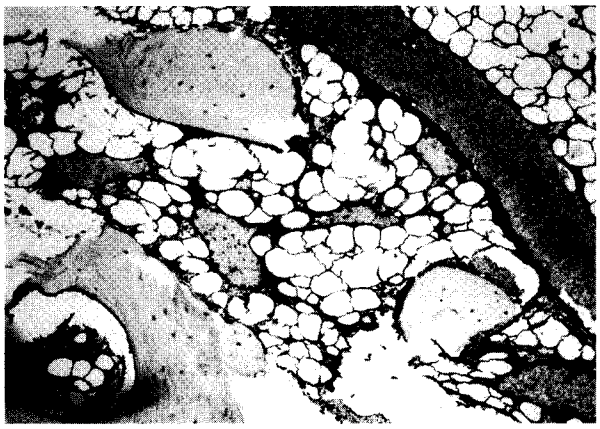

Fig. 1. TGF-b_{1r} monoclonal antibody staining shows cancellous marrow grafts to possess cells bearing receptors for TGF-b_1 growth factors; they are more concentrated around vessels (perivascular loci) and on endosteal osteoblasts. TGF-b RI (V-22) epitome correspond to amino acids 158-179 (Santa Cruz Biotechnology stains, original magnification

cm or greater arising from benign and malignant tumor extirpations without radiotherapy were randomized into two groups. One group received cancellous cellular marrow grafts without added PRP. The second group received grafts with PRP added during the bone-milling phase of graft preparation and applied topically after bone placement into the defect. For each graft the posterior ilium was used as a donor site.

The PRP was obtained by means of an Electro Medics 500 gradient density cell separator (Medtronics) used in the operating room simultaneously with bone graft harvesting. This cell separator withdraws 400 to 450 ml of autologous whole blood through a central venous catheter placed during surgery. With a centrifuge speed of 5600 RPM, whole blood is drawn at a rate of 50 ml/min. As it withdraws the blood the separator adds citrate phosphate dextrose (CPD) at a ratio of 1 ml of CPD to 5 ml of blood to achieve anticoagulation. The blood is then centrifuged into its three basic components; red blood cells, PRP (sometimes referred to as "buffy coat"), and platelet-poor plasma (PPP). Because of differential densities, the red blood cell layer forms at the lowest level, the PRP layer in the middle, and the PPP layer at the top. The cell separator incrementally separates each layer, from the less dense to the more dense; therefore it separates PPP first (about 200 ml) and PRP second (about 70 ml), leaving the residual red blood cells (about 180 ml). Once the PPP is collected, the centrifuge speed is lowered to 2400 RPM to allow for a precise separation of the PRP from the red blood cells. In fact, both our experience and testing by Reeder et al.4 have shown that the platelets most recently synthesized, and therefore of greatest activity, are larger and mix with the upper 1 mm of red blood cells, so that this layer is included in the PRP product. This imparts a red tint to the PRP, which would otherwise be straw colored. The red blood cells and PPP are returned to the patient from their collection bags through either the central venous catheter or a peripheral venous access.

This procedure takes approximately 20 to 30 minutes. However, it is accomplished simultaneously with either the bone harvesting procedure or preparation of the recipient tissues, and therefore it does not add to operating room time. The Medtronics cell separator is in the armamentaria of most operating rooms that are also used for major orthopedic and cardiovascular surgery; there are thus no additional expenses except those associated with disposable catheters, a central venous line, and an internal centrifuge bowl, which together cost approximately $300.00.

Samples of PRP and venous blood were submitted for machine platelet counts and a smear with Giemsa staining for a manual count. Two additional PRP smears were stained with monoclonal antibody stains (Santa Cruz Biotechnology, Santa Cruz, Calif.). One was stained for PDGF and the other for TGF-b. A sample of the autogenous graft material was placed in formalin, processed with a slow formic acid decalcification, and stained with monoclonal antibodies to identify PDGF receptors ($PDGF_r$) and TGF-b receptors ($TGF\text{-}b_r$).

The PRP application requires initiating the coagulation process with a mixture of 10 ml of 10% calcium chloride mixed with 10,000 units of topical bovine thrombin (Gentrac). The protocol for PRP application requires the use of an individual 10-ml syringe for each mix. Each mix draws, in order, 6 ml of PRP, 1 ml of the calcium chloride/thrombin mix, and 1 ml of air to act as a mixing bubble. The syringe is agitated for 6 to 10 seconds to initiate clotting. The PRP, now a gel, is added to the graft in several mixes. If several mixes are used, a sterile new syringe is required at each mix. The addition of a small amount of calcium chloride and thrombin from a reused syringe can coagulate the remainder of the PRP in its container. Once the PRP is added to the graft the fibrin formation binds the otherwise loose cancellous cellular marrow together to assist the surgeon in sculpting the graft. The fibrin network established in the graft is thought to assist the osteoconduction component of bone regeneration.[1]

The bone grafts were allowed to consolidate and mature for 6 months. Panoramic radiographs were taken at the 2-, 4-, and 6-month intervals. The unlabeled panoramic films were assessed by two investigators (S.R.S. and R.E.M.) as to the age of the graft at

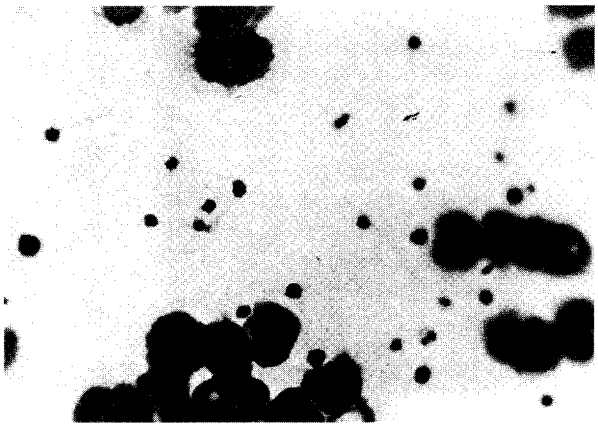

Fig. 2. Normal platelet density in peripheral blood smear (Giemsa stain, original magnification ×10).

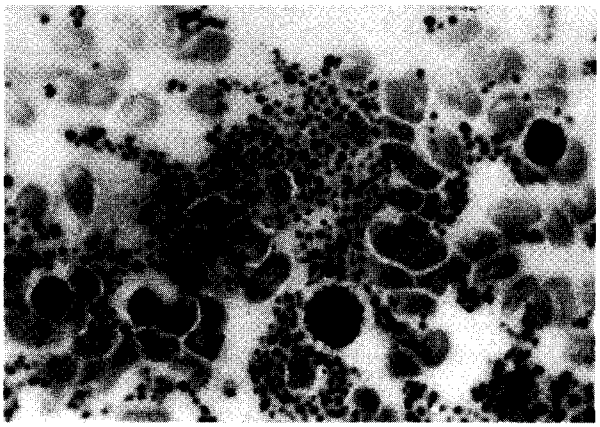

Fig. 3. Obvious concentration of platelets seen on PRP smear indicates transplantation of additional platelets into graft. Cytoplasmic granules (alpha granules) are known location of PGGF, TGF-b_1, and TGF-b_2, among other growth factors.

Table I. Platelet counts: 338% increase

Baseline platelet count	*PRP platelet count*
232,000 (111,000-523,000)	785,000 (595,000-1,100,000)

each interval. The ratio of assessed graft maturity to actual graft maturity gave a numeric index of graft maturity (graft maturity index [GMI]). At least one osseointegrated implant was placed into each graft at the 6-month interval. The placement of implant fixtures through use of a /3i implant (Implant Innovations, Inc.) with a diameter of 4.0 mm allowed a core bone specimen 2.9 mm in diameter to be processed for histomorphometry and for monoclonal antibody staining for $PDGF_r$ and TGF-b_r. Histomorphometry was accomplished with a semiautomatic computer image system (SMI Unicomp, Atlanta, Ga.). This system projects the histologic image onto a video screen. Random areas were traced on a digitizing pad to calculate area of mineralized bone matrix versus total area of view. The area of mineralized bone matrix was recorded as trabecular bone area (TBA) versus marrow space area. For purposes of comparison and control, 10 resection specimens of the midbody of the mandible were assessed with the same histomorphometric technique, and a TBA was calculated for each.

RESULTS

PRP monoclonal antibody study

The platelets sequestered by the centrifugation process showed an intense uptake of both PDGF and TGF-b monoclonal antibodies in all slides, thus confirming the presence and retention of these growth factors in the PRP preparation.

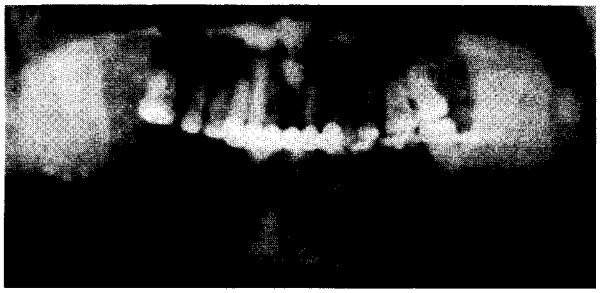

Fig. 4. Functioning cancellous marrow graft where PRP was not used is well consolidated at 6 months.

Bone graft harvest material monoclonal antibody study

All slides of harvested cancellous cellular marrow showed cell populations that tested positive for receptors to PDGF and TGF-b. It was observed that most of these cell populations were centered about blood vessels in a perivascular sheet. Lesser numbers were observed on the trabeculae of the cancellous bone's endosteal surface and randomly dispersed between fat cells in the marrow. (Fig. 1). These results identified the presence of marrow stem cells and osteoprogenitor cells within human cancellous marrow capable of responding to the increased PDGF and TGF-b in the PRP preparation.

Platelet count study

Platelet counts done on each patient yielded a mean platelet count value of 232,000, with a range of 111,000 to 523,000. The PRP mean platelet count was 785,000, with a range of 595,000 to 1,100,000. These values confirmed the platelet

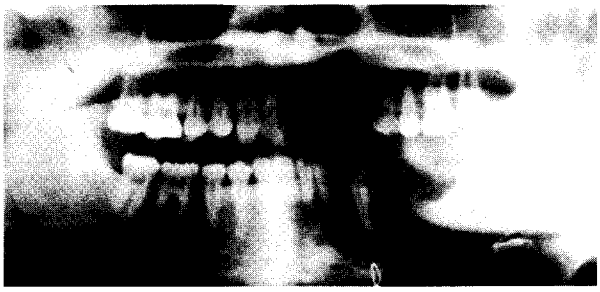

Fig. 5. Functioning cancellous marrow graft where PRP was used shows an enhanced maturity and bone consolidation at 6 months.

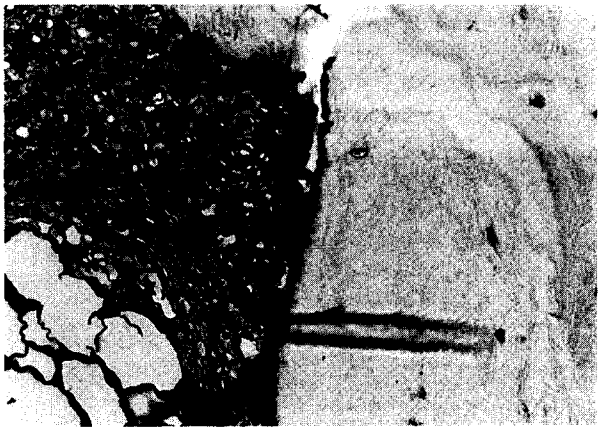

Fig. 6. TGF-b_{1r} monoclonal antibody staining shows cell producing TGF-b, presumably to maintain bone formation in graft as an autocrine stimulation. TGF-b_{1r} (v) epitome corresponds to amino acids 352-377 (Santa Cruz Biotechnology

sequestration ability of the process and quantified the concentration as 338% of baseline platelet counts (Table I; Figs. 2 and 3).

Assessment of radiographic graft maturity

The results of the panoramic radiographic assessment are illustrated in Table II. At 2 and 4 months the grafts without PRP growth factor additions were assessed at or just below their actual maturity; at 6 months they were assessed at or just ahead of the actual graft maturity. The grafts with PRP growth factor additions were consistently assessed either at or at slightly more than twice their actual maturity, with ratios of 2.16 at 2 months, 1.88 at 4 months, and 1.62 at 6 months. Each comparison of the average graft maturity index values of PRP-added grafts with those of the grafts without PRP was assessed by means of a Student *t* test. The *p* value for each comparison was 0.001 (Figs. 4 and 5).

Fig. 7. Trabecular bone area of human posterior mandible. Mean trabecular bone area is 38.9% ± 6%.

Table II. Graft maturity index

Time (mo)	*Grafts*	*Graft + PRP*	P
2	0.92	2.16	0.001
4	0.88	1.88	0.001
6	1.06	1.62	0.001

Table III. Histomorphometric findings at 6 months

	TBA	P
Native mandible (10)	38.9% ± 6%	-
Bone grafts (44)	55.1% ± 8%	0.005
Bone grafts with PRP (44)	74.0% ± 11%	0.005

Six-month graft assessment with monoclonal antibodies

Processed core bone specimens of each graft type at 6 months demonstrated a continued production of TGF-b. Monoclonal antibodies identified TGF-b but not PDGF by marrow stem cells and endosteal osteoblasts. The TGF-b–positive cells were noted to be concentrated on the trabecular bone endosteal surface, on the periosteal surface, and within active marrow stem cells. Only rare cells stained positive for PDGF and were thus interpreted as nonreactive (Fig. 6).

Six-month graft assessment with histomorphometry

The results of the histomorphometric study, which are illustrated in Table III, indicated that bone grafts in general produce a trabecular bone area greater than that of native posterior mandible (55.1% ± 8% vs 38.9% ± 6%; $p = 0.005$). This was not unexpected and has been reported earlier.[2,3] However, bone grafts with growth factors added by means of PRP demonstrated even greater trabecular bone density than did bone