ORIGINAL ARTICLES

Platelet-Rich Plasma (PRP) Utilized To Promote Greater Graft Volume Retention in Autologous Fat Grafting

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?1?2 ***Objectives:*** *Autologous fat theoretically provides one of the most ideal mediums for soft-tissue augmentation and reconstruction, although its clinical applications have been marked with skepticism because of its documented unreliable survival. Over the years, numerous unsuccessful efforts have set forth to elucidate modifications in the application process of autologous fat grafts to allow the medium greater clinical predictability. This study aims to investigate the effects of platelet-rich plasma (PRP) on autologous fat grafts when used in conjunction with each other in soft tissue augmentation and reconstruction.*

Study Design: *Retrospective review, over a 30-month period, of consecutive patients with results greater than 6 months in duration.*

Methods: *This study is based on clinical experiences representing 2033 grafts in 448 consecutive patients using PRP additives and in the previous 132 patients who had syringe harvest without use of PRP. All PRP isolates were harvested via the Smart Prep system. Harvest and augmentation techniques are discussed and representative results are presented.*

Results: *Results were based on clinical observations and patient satisfaction. Of the 580 patients in the experimental group, essentially all showed greater graft volume retention over extended time intervals compared with control subjects (nongraft areas). Patients in the PRP-added experimental group displayed less postoperative ecchymosis and edema, which also led to greater patient satisfaction in this group.*

Conclusion: *Adding PRP to autologous fat aids in graft volume retention and survival when used clinically for soft-tissue augmentation and reconstruction.*

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The selection of autologous graft materials is widely accepted as one of the most fundamental mediums for use in most soft-tissue augmentation and reconstruction dilemmas. It provides a very versatile augmentation medium for cosmetic and reconstructive surgeons. Adipose tissue provides a readily available, autologous graft medium for which use in human autotransplantation has been documented for more a century.[1,2] Autologous fat affords a medium that is ?3 soft, pliable, and readily available in abundant stores; can be harvested with minimal morbidity; has low antigenicity; and lacks risk of disease transmission.[2,3–5] In light of the aforementioned benefits, the use of autologous fat as a graft medium has been fraught with skepticism by the cosmetic surgery community. This skepticism lies in the relatively inconsistent and unpredictable survival rates of autologous fat grafts to date. These results frequently necessitate the need for overcorrection of soft-tissue volume defects and increase the possibility of multiple procedures to achieve the desired volume of augmentation and symmetry. Because of these unpredictable outcomes, many studies have focused on modifying various parts of this procedure to achieve greater graft survival rates.[6,7] Several studies have sought to modify and ?4 standardize the harvest procedures, whereas others have tried to provide additives that might improve graft survival. Unfortunately, many of these attempts have fallen short of their goals. Over the past decade, a better understanding of the biochemical milieu of the wound-healing process has enhanced the ability to assist healing.

This project is focused on studying the effects of enhancing fat-graft survival by augmenting the biochemical healing potential of the graft material with the addition of platelet-rich plasma (PRP, also known as autologous platelet concentrate). PRP maintains a high

concentration of bioactive proteins and growth factors that are shown to precipitate and augment tissue repair and regeneration processes.[8–19] Results of clinical trials have suggested that growth factors not only influence the viability of transferred cells but also may play a bioactive role in influencing the differentiation of precursor adipocytes within the graft into their mature form.[4,20–24] Clinical trials have documented the efficacy and safety of the use of such concentrates in hard- and soft-tissue augmentation by stimulating and enhancing the native repair and regeneration of osseous and soft tissues.[3,14,15,17,25,26] That evidence has already been clinically reviewed, so this study seeks to further report on the clinical improvements noted in the effects of autologous platelet concentrates with regards to the predictability and effective viability of autologous fat as a soft-tissue augmentation medium.

Definition of PRP

PRP describes a volume of autologous plasma that has a platelet concentration typically 5 or 6 times the normal baseline levels. PRP is isolated from an autologous whole blood sample by a process of differential centrifugation.[27] PRP can be applied to wound sites directly in its isolated form or in form of a platelet gel created by initiation of the coagulation process and adding thrombin and calcium chloride.
?5 PRP is defined as 1,000,000 platelets/[mu] in a 5 mL volume of plasma, which is the concentration at which bone and soft-tissue healing enhancements have been scientifically reported.[16,27] This high concentration of platelets, as well as the component parts, is what allows PRP to become a strong bioactive element, providing high concentrations of growth factors contained predominantly within the alpha granules of its platelets to enhance wound healing.[16,17] The major documented growth factors contained in PRP include platelet-derived growth factor (PDGF) aa, PDGFbb, PDGFab, transforming growth factor β-1, transforming growth factor β-2, vascular endothelial growth factor, and epithelial growth factor.[16,17]

Materials and Methods

Isolation of PRP

Isolation of autologous platelet concentrates was once a cumbersome process, requiring expensive equipment and technical staff to isolate and prepare such materials for use in surgery. With the advent of affordable equipment and kit development, perioperative isolation of PRP is easily and safely completed at outpatient bases, using an automated dual-spin process (SmartPreP, Harvest Technologies, Inc, Plymouth, Mass).

Closed Syringe Harvest of Autologous Fat

Most surgeons who are experienced at fat transfer have adopted use of low pressure, syringe harvesting of fat-graft materials. In the authors' practice, harvesting is carried out using tumescent fluid infiltration of the donor sites composed of 0.05% xylocaine with 1:1,000,000 epinephrine. In this study, use of tumescent volume of infiltration was at a ratio of 2:1 (fluid to supranatant graft), and there was an attempt to gently extract the graft materials via Cell Friendly (Tulip BioMed, San Diego, Calif) microcannulas using a technique that was as minimally traumatic as possible. Efforts were made to minimize graft trauma including using polished blunt cannulas of a somewhat larger diameter (2.0–3.0 mm), displacing air from the system before use with sterile saline or Ringer lactated solution. During harvest, low pressure is applied by limiting the plunger movement to half or less of the syringe being used. The Tulip Cell Friendly System was selected based on the internal superpolished lumens and smoothed exterior for graft collection with minimal trauma. In the authors' experience, use of slightly larger harvest tubes, minimal-extraction vacuum pressures, and superpolished titanium cannulas provides the most atraumatic means for graft harvest.

After fat harvesting, the graft was serially rinsed to reduce the residual intracellular lidocaine and debris (including cellular remnants, blood products, free lipids). At this point the graft is ready for the addition of autologous platelet concentrates in a 10% concentration PRP to rinsed graft.

Closed Syringe Harvest of Autologous Fat

Autologous Fat Graft Preparation with PRP

The PRP is first added to the prepared fat graft in a ratio of 1:9 (10%), and after gentle agitation it is left undisturbed for 10 minutes to permit release of the platelet-concentrate component elements. After the 10-minute interval the graft material is ready for injection. The prepared fat-graft material is then placed in various size injection syringes (1–10 cm^3 luer-loc syringe) with polished Cell Friendly transfer needles ranging from 1 mm to 2.1 bore cannulas for most small volume transfers, and 1.7–3.0 bore for large-volume grafting ?6 procedures. In all instances, gentle pretunneling of the recipient sites should be performed in layers to prepare

Table 1. Total Grafts Performed (% by Location) at 2033 Graft Sites

Grafting with PRP*	Grafting without PRP (n = 919) (%)	Grafting with PRP (n = 1114) (%)
Malar/submalar	29%	32%
Nasolabial folds	18	10
Malar-facial grooves	5	10
Lip vermillion	12	14
Depressed scars	5	2
Infracommissures	6	5
Chin	4	3
Mandibular body-angle	3	3
Pre-jowl depressions	3	2
Trunk & Extremities	15	19

*PRP indicates platelet-rich plasma.

a recipient bed to accept the small micrografts laid in the developed space under minimal pressures.

Results

This study is based on clinical experiences representing 2033 grafts in 580 consecutive patients treated from
?7 January 2002 through June 2004; PRP additive was used in 448 patients, and 132 patients had no PRP additive. Each group used low-pressure closed syringe harvest with Cell Friendly cannulas, a minimum of 2 saline rinses, no centrifugation, and no use of osmotic
?30 stabilization agents (such as albumin) in either group.

Clinical observations and patient satisfaction review of cases using autologous platelet concentrate (PRP) as an additive to autologous fat grafts suggests that this method of soft-tissue augmentation may have clinically significant advantages over conventional fat-grafting techniques. PRP-enhanced grafts appear to show a greater potential for graft acceptance and retention over some existing conventional techniques as documented by physician findings and patients' clinical satisfaction levels. Clinical results have been very encouraging from the standpoint of greater graft-volume retention. It has also been noted that there appears to be less swelling and bruising at the donor sites after injection compared with patients who underwent conventional techniques without addition of PRP.

Figure 3 displays a female patient 1 year after autologous fat grafting with PRP to the nasolabial folds and lips. This patient continues to display marked rejuvenation in the areas noted. There continues to be noticeable improvements: continued fullness of the lips, some reduction of perioral rhytids, enhanced

Figure 1. *Isolation of PRP. Stage 1-Transferring whole blood into blood chamber. Stage 2-Load SmartPReP.® Stage 3-After processing, Platelet Poor Plasma (PPP) is isolate (Yellow). State 4-Then Platelet Rich Plasma (PRP) is isolated (red). Photos courtesy of Harvest Technologies. Closed Syringe Harvest of Autologous Fat.*

definition of the cupid's bow, and reduced prominence of the nasolabial folds. Furthermore, there are no palpable nodules at the recipient sites, and the patient has not required further follow-up procedures.

Figure 4 displays a female patient 1 year after rhytidectomy and autologous fat grafting with PRP to the malar fat pads and nasolabial folds. One year after treatment the patient continues to display noticeable fullness and definition of the malar fat pads with continued reduction of the nasolabial folds.

Similar results can be seen in Figure 5, which shows a woman 1 year after rhytidectomy and autologous fat grafting with PRP to the malar fat pads and nasolabial folds. This patient also continues to display youthful definition in the cheeks and continued reduction of the nasolabial folds.

Figure 6 shows a woman 6 months after autologous fat grafting to the lips. The patient displays a continued fullness of the upper and lower lips. Although this is only a 6-month follow-up, the patient will likely continue to be observed to mark progress and effects.

Figure 7 shows a woman 2 years after autologous fat grafting to the breast. The patient displays a continued fullness. Although there was much improvement in breast fullness, she is receiving more fat graft for slightly larger breast at a second stage. Mammographies preoperatively, at 6 months, and then annually have revealed no abnormalities or cystic calcifications.

Figure 2. *Addition of PRP Isolate to Graft Material.*

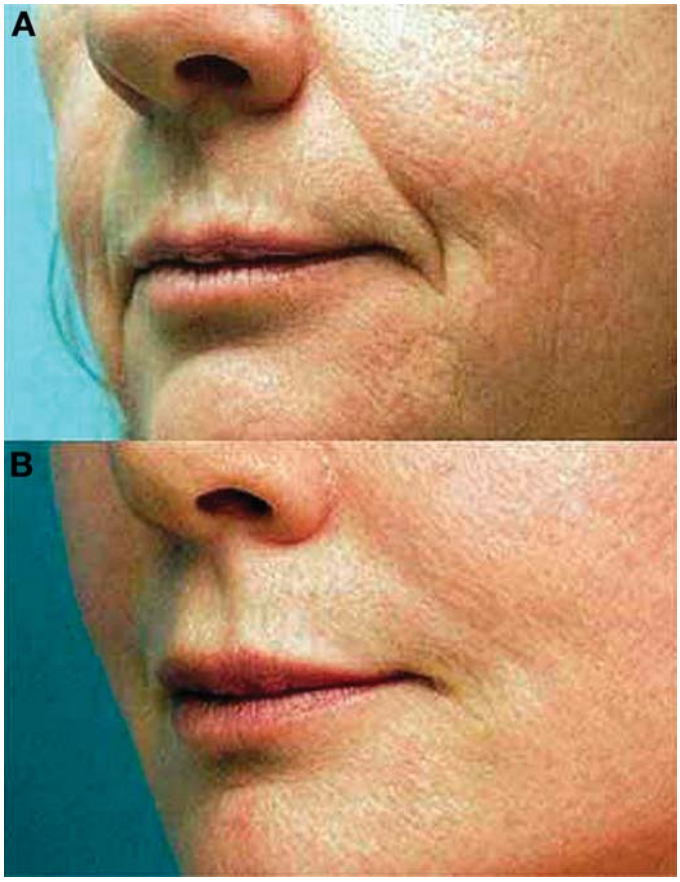

Figure 3. *A: Preoperative photograph. B: One year after autologous fat grafting with PRP to nasolabial folds and lips.*

Although the current results were based on qualitative, clinical findings, efforts are being set forth to further study the procedure from a quantitative, cellular level.

Discussion: The Fat-Graft Healing Model

?31 The literature contains numerous studies that have sought to elucidate a biochemical additive or agent that can improve the acceptance and outcome of autologous fat grafts. Numerous additives, such as heparin, calcium, thyroid hormone, bezafibrate, and vitamin E have been studied with little or no evidence supporting greater graft acceptance.[1,3,7,22] Growth factors are the biologically active signal peptides released from local tissue or blood products that play a critical role in influencing the initiation and progression of the normal wound-healing process.[3,12–14,16,17,25,27,28] Growth factors coordinate the processes of epithelialization, angiogenesis, and collagen/matrix formation, which are the key steps in wound healing.[13] Growth factors function in paracrine, endocrine, and autocrine manners to guide the dynamic stages of wound healing.[13] No single growth factor appears to maintain a specific physiologic task; instead, these peptides work in a coordinated fashion to orchestrate the normal wound-healing process.[13] A substantial amount of research has displayed the efficacy of these bioactive peptides with regards to healing in both soft and osseous tissue.[11,12,25,26,29] In a randomized, prospective, double-blind, placebo-controlled study of 118 patients with chronic, full thickness, lower extremity diabetic neurotrophic ulcers of at least 8 weeks, there was a statistically significant difference in numbers of patients healed and healing rates after daily application of topical recombinant growth factor.[30] Forty-eight ?8 percent of the patients randomized to the growth factor application group achieved complete wound healing, compared with only 25% who achieved wound healing in the placebo group. Knighton et al[18] displayed 93% re-epithelialization in 41 patients displaying a total of 71 chronic wounds after receiving daily treatments with autologous platelet concentrate. In a second study, Knighton et al[19] reported the re-epithelialization of 17 of 21 chronic lower extremity ulcers that were treated

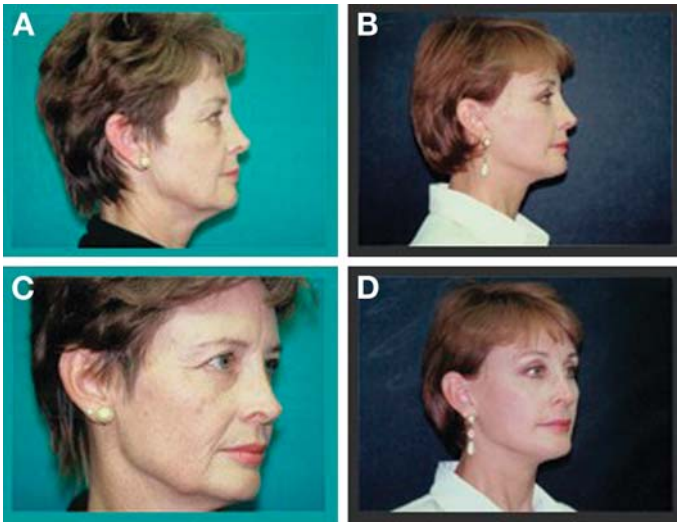

Figure 4. *A. Preoperative photograph. B. Patient at one year status postrhytidectomy and autologous fat grafting with PRP to the malar fat pads and nasolabial folds.*

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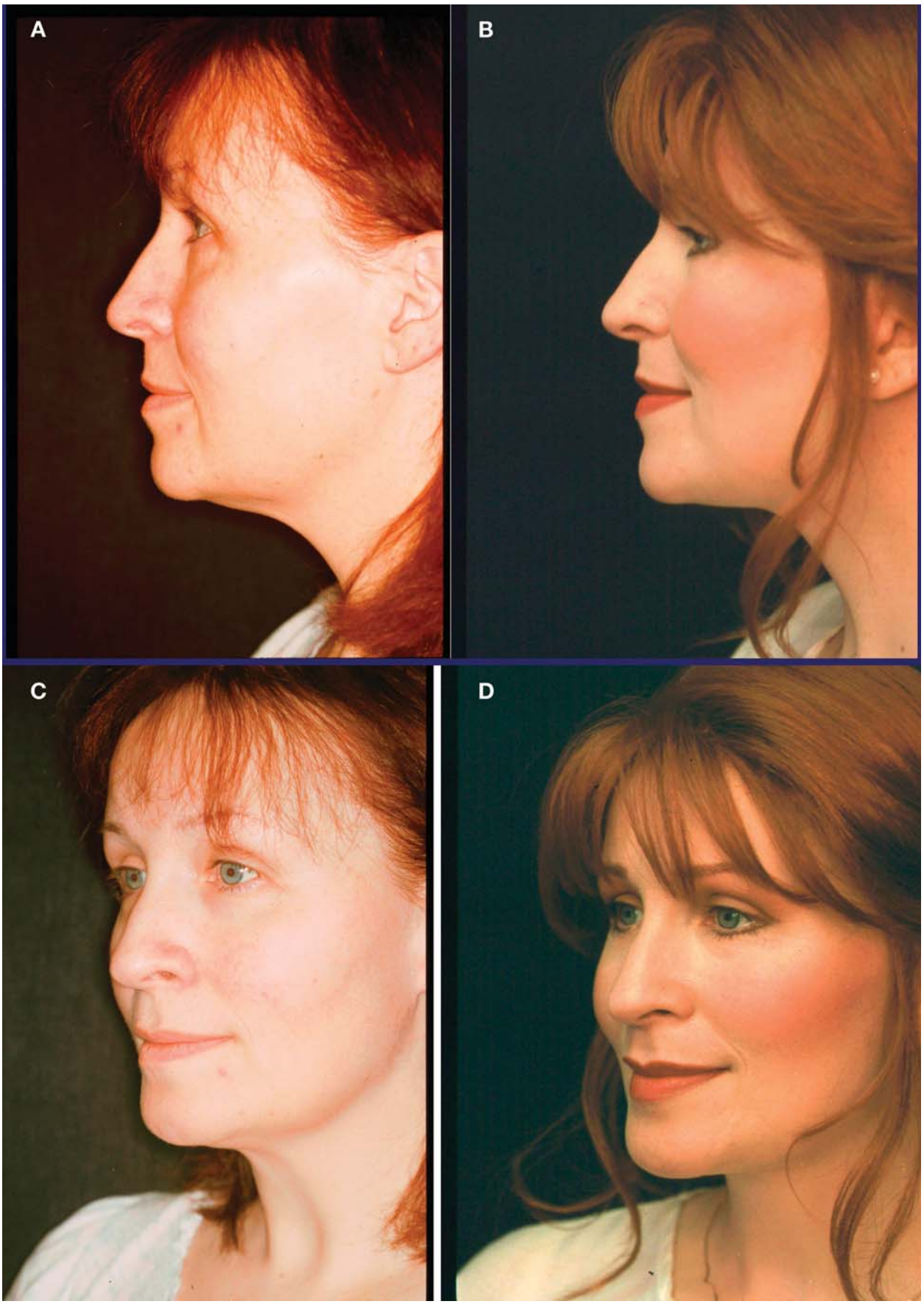
Figure 5. *A: Preoperative photograph. B: Patient at one year postrhytidectomy, autologous fat grafting to malar fat pad with PRP.*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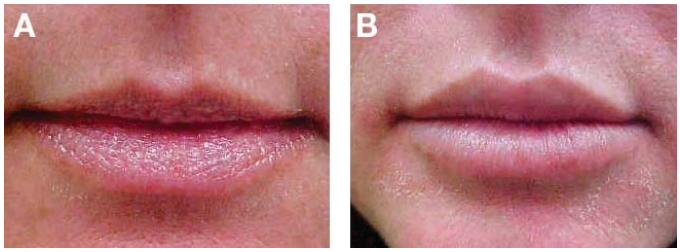

Figure 6. *A: preoperative photograph. B: Patient at six months after autologous fat grafting with PRP to lips.*

with an 8-week course of twice daily autologous platelet concentrate. Of the patients in the placebo group, only 2 of 13 their wounds displayed the same results. Upon crossover treatment of the placebo group with autologous platelet concentrate, all previously unresponsive wounds displayed re-epithelialization. ?9 Ganio et al[15] displayed a 78% limb salvage rate among a series of 171 patients with a total of 355 wounds of average 75 weeks' duration after daily treatment with a platelet-derived wound-healing factor concentrate for an average of 10 weeks. In the field of oromaxillofacial ?10 surgery, Marx et al. reported enhancement of bone formation on bone biopsy specimens in mandibular bone grafts after treatment with PRP. Relevant to facial plastic surgery, Powell et al reported trends that may ?11 suggest enhancement of recovery with decreases in postoperative edema and ecchymoses in a pilot, randomized, prospective, controlled clinical trial involving 8 female patients treated with autologous platelet gel during standard deep-plane facelift. The effect of growth factors on enhancement of neovascularization was studied by Khouri et al.[31] This study demonstrated that after the application of basic fibroblast growth factor to ischemic flaps in rat models, the experimental group displayed greater flap survival rates, as well as a greater increase in the number of new blood vessels upon histologic examination.[31]

PRP, a clinically documented promoter of the ?32 wound-healing process, contains supraphysiologic concentrations of growth factors. It is the intention of the present study to extrapolate the basic wound-healing model with respect to the transplantation of autologous fat and to study the effects of autologous platelet concentrates within this environment. It is postulated that the effects of PRP, with regard to enhancement of normal tissue healing processes, can be safely used as an additive in autologous fat transplantation to promote

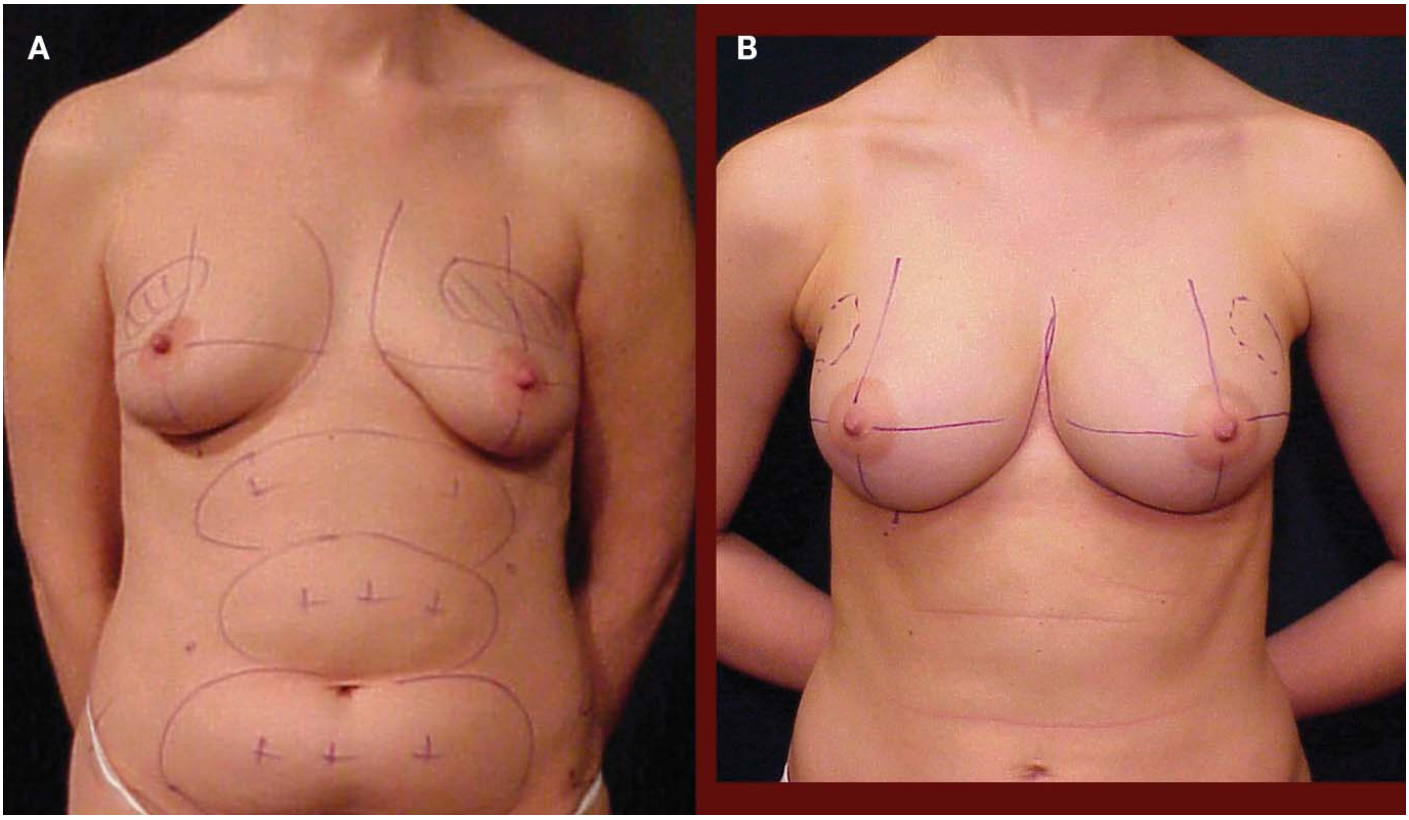

Figure 7. *A: preoperative photograph. B: Patient at two years after autologous fat grafting with PRP to breast.*

Table 2. Frozen Grafts Versus Fresh Grafts

Frozen grafts		
With PRP*	235/2033	
Without PRP	149/2033	384/2033 = 19% of total (frozen)
Fresh harvest grafts		
With PRP	1114/2033	
Without PRP	919/2033	1649/2033 = 81% of total (fresh)

*PRP indicates platelet-rich plasma.

Table 3. Results of Patient/Surgeon Satisfaction Survey Administered 6 Months to 1 Year After Procedure*

Rating	With PRP† (n = 448)	Without PRP (n = 132)
	Patient/Surgeon	Patient/Surgeon
Excellent	11%/12%	4%/5%
Better than expected	26%/30%	10%/11%
As expected	53%/51%	36%/59%
Less than expected	8%/6%	31%/21%
No change	2%/1%	19%/4%
Would do again‡	85%	51%
Would recommend‡	85%	50%
Would not do again‡	10%	33%
Did not respond‡	5%	16%

* Total number of grafts performed was 2033.
†PRP indicates platelet-rich plasma.
‡No surgeon used this rating.

increased graft volume retention and return to metabolic activity. Adding PRP (and the attendant addition of high concentrations of growth factors and cytokines) may increase the retention of the transplanted fat cells, increase the rate of revascularization of the graft, and aid in the differentiation of preadipocyte precursor cells into mature adipocytes to further augment graft volumes. Use of mesenchymal stem cells derived from fat is the current subject of study, as they appear to be able to undergo induction differentiation and are more prevalent and easier to obtain than bone marrow cells.

In the case of autologous fat transplantation, the recipient bed represents the basic model of soft tissue injury. The authors believe there is clinical enhancement of wound repair in the recipient bed, through the effects of adding PRP, that appear to favorably aid in graft-volume retention and graft acceptance at site. Autologous fat cells are injected into a created potential space that has been subjected to tissue injury as a result of pretunneling at the recipient site. After grafting, this site consists of transplanted cellular elements, clotted blood containing platelets, exposed endothelial cells, interstitial collagen, fibroblasts, and undifferentiated stem cell elements.[3]

It is suggested that this model represents the initiation of the wound-healing process. With the occurrence of tissue injury, the damaged recipient bed cells (native) release growth factors (notably PDGF and transforming growth factor).[13,32] Concurrently, activation of the clotting cascade results in the transformation of fibrinogen to fibrin, a potent stimulus for the release of growth factor from the alpha granules within the aggregated platelets.[13,32] These growth factors then activate their target cells (polymorphonuclear neutrophil leukocytes, macrophages, lymphocytes, fibroblasts, endothelial cells, epithelial cells) to proliferate and migrate.[32] Macrophages secrete growth factors that induce fibroblast proliferation and collagen synthesis, endothelial cell replication and mobilization, and epidermal cell mobility and proliferation.[13,32] Angiogenic growth factors are released by the injured cells, platelets, macrophages, and extracellular matrix, inducing vascularization.[13] It is evident that growth factors play a key role in initiating, expediting, and coordinating the phases involved in wound healing. The investigators of this study believe adding PRP, which contains supraphysiologic concentrations of the growth factors necessary for normal wound healing, may help to markedly augment and improve the healing process at the recipient bed. This enhancement of healing rate and graft survival may result in more favorable and reliable results with regards to success in transplanting autologous fat.

It is postulated that the growth-factor enhancement seen in general wound healing may play a key role in allowing greater survival of transplanted adipocytes. Evidence in the literature suggests that growth factors may play a role in initiating the differentiation of adipose precursor cells into mature adipocytes.[3,4,20–24] Eppley et al,[20] in a later study, observed the effects of basic fibroblast growth factor on fat grafts at up to 1 year after grafting. The results showed near complete graft-weight maintenance, larger adipocyte volume, increased numbers of intact cells, and the presence of numerous smaller adipocyte-like cells compared with controls 1 year after grafting.[20] These results suggest that growth factors may enhance graft retention volumes and increase the number of adipocytes within the grafted tissue as evidenced by the increase in mean adipocyte area percentages in the experimental groups.[20] These studies suggest that adding growth

factor to autologous fat grafts before transplantation may aid in improving fat-graft survival by influencing differentiation of pre-adipocyte cells contained in the graft tissue. Thus, adipocytes that may be lost in the transplantation process may be replaced by new cells, which allows the overall graft volume to be maintained. Further, many conflicting reports estimating volume survival in fat grafting seem to ignore the fact that the harvest and transfer processes involve a fat cell suspension including 20–40% fluid components. In determining clinically successful graft volume, the fluid component should be expected to be gradually reduced during the healing processes. Claims that fat grafting does not work, or that it shows categoric loss of 30–50%, should be evaluated carefully, while accounting for the extracellular fluid carrier volumes in final volume retention estimates.

Conclusion

Correction of soft-tissue defects continues to be one of the major challenges for cosmetic and reconstructive surgeons. Autologous fat theoretically provides a near-ideal medium for soft-tissue augmentation. It is a medium that is readily available in abundant quantities, has good physical properties, can be harvested with relative ease and low morbidity, and provides a graft medium with little antigenicity.[2–5] The one factor that has frustrated many aesthetic surgeons has been the relative 20–50% loss of site volume after transplantation.[4] Studies have sought to elucidate new methods or additives that could prevent this loss, although all have provided little scientific evidence to quantitate the successful grafting of autologous fat. The emergence of growth-factor technology and in vitro evidence has shed promising light on autologous fat grafting. Studies have shown results that may support the role of growth factors in providing greater autologous fat graft volume retention.[1] It is also believed that adding growth factors to the graft medium may also stimulate differentiation of adipose precursor cells into their mature form, which would further maintain graft volumes after transfer.[4,20–22] Additional study in this area is certainly warranted.

The present study has sought to describe a safe and effective protocol to isolate and use autologous platelet concentrates, which contain much greater concentration than normal wound-site concentrations of growth factors, in an effort to potentially improve the survival and clinical outcomes of autologous fat grafting in the body. The rationale for using PRP as an additive in the transfer process was to create an optimal microenvironment in the recipient bed that would help to expedite and enhance the wound-healing process and graft incorporation. The authors believe PRP may provide the ideal additive to autologous fat grafts to enhance their reliability and clinical success. This study suggests that more standardization and investigation is needed in the areas of small- and large-volume transfer. The authors are currently studying the potential of transfer or activation at recipient site from the mesenchymal stem cell components found in the fat matrix. It has been documented that more mesenchymal stem cells are available in fat tissues than in bone.[19] With the advent of many investigators reporting successful large-volume micrograft augmentation in the buttock and breast areas, additional interest in large-volume transfers using PRP has been forthcoming. Further investigation is needed in the form of standardization and accumulation of data from a large-scale, multi-institutional study. Research that provides methods to successfully and uniformly quantify survival and metabolic activities of graft tissue will help determine the ideal clinical application of the techniques proposed in this study.

In conclusion, this study suggests that autologous platelet concentrate/PRP may provide great clinical potential for autologous fat transplantation and that there is a safe and economical way to isolate it for use in a wide variety of general, orthopedic, cosmetic, and reconstructive surgeries.

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