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Abstract

Background. The purpose of this study was to review the authors' experience of fat grafting, evaluating the effects related to the use of fat grafting with platelet-rich plasma (PRP) in the improvement of fat volume in breast reconstruction and comparing the results with a control group (only centrifuged fat grafting). **Methods.** A total of 50 patients aged between 19 and 60 years affected by breast soft-tissue defects were analyzed at the Plastic and Reconstructive Department of the University of Tor Vergata. They were treated with fat grafting + PRP. The control group (50 patients with breast soft-tissue defects) were treated with centrifuged fat grafting injection according to Coleman's procedure. **Results.** The patients treated with PRP added to the autologous fat grafts showed a 69% maintenance of the contour restoring and of 3-dimensional volume after 1 year, whereas the patients of the control group treated with centrifuged fat grafting showed a 39% maintenance. **Conclusion.** PRP mixed with fat grafting leads to an improvement in maintaining breast volume in patients affected by breast soft-tissue defects.

Keywords

platelet-rich plasma, fat graft with platelet-rich plasma, breast fat graft, PRP

Introduction

Breast reconstruction with autologous fat graft has become common in recent years. Many authors have contributed to the development of the technique, adding to the fat the stromal vascular fraction cells or growth factors. This technique was applied not only for breast defects but also for other reasons: radiotherapy-based tissue damage after mastectomy,¹ breast augmentation,² postmastectomy breast reconstruction,³ breast implant complications,⁴ calvarial defects,⁵ Crohn's fistulas and complex perianal fistula,⁶⁻⁹ damaged skeletal muscle,¹⁰ Perry-Romberg disease, and facial lipoatrophy,¹¹ scarring, gluteal soft-tissue defect, pectus excavatus, dermatofibrosis,¹² and vocal fold augmentation.^{13,14}

Recently, we published the results of studies on the use of fat grafting in the lipostructure technique, as described by Coleman,^{15,16} where it is mixed with platelet-rich plasma (PRP),¹⁷ in lower chronic extremity ulcers¹⁸ and in hemifacial atrophy.¹⁹ In this article, we present our experience using PRP mixed with fat grafting. Patient self-assessment of the outcomes is an additional parameter supporting the results of clinical assessment.

Methods

Patients

A total of 100 patients aged between 19 and 60 years were treated from January 2008 to January 2012 at the Plastic and Reconstructive Surgery of the University of Tor Vergata, Rome, Italy. The patients were divided into 2 groups. Group A consisted of 50 patients affected by breast soft-tissue defects (including 10 patients affected by unilateral breast hypoplasia, 30 patients affected by outcomes of breast cancer reconstruction, and 10 patients affected by outcomes of prostheses removal) who were treated with fat graft + PRP. The patients were not affected by relevant diseases and were free from cancer for 5 years at least. The control group consisted of 50 patients (including 5 patients affected by unilateral breast hypoplasia, 35 patients affected by outcomes of

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breast cancer reconstruction, and 10 patients affected by outcomes of prostheses removal) who were treated with autologous fat grafts only.

For each group, preoperative evaluation was performed, which included a complete clinical examination, photographic assessment, nuclear magnetic resonance imaging (MRI) of the soft tissue, and ultrasound. In more complex cases, as in the case of absence of pectoralis muscle and Poland Syndrome, a high-resolution CT scan with 3D imaging was performed. Postoperative radiological follow-up was performed at 2, 6, 12, and 24 months and then annually. Postoperative clinical follow-up took place at 2, 6, 12, 21, and 36 weeks and then annually.

Exclusion criteria were divided into 2 types: local and systemic. The systemic criteria included platelet disorders, thrombocytopenia, antiaggregating therapy, bone marrow aplasia, uncompensated diabetes, sepsis, and cancer. The local criteria included cancer and loss of substance. We did not consider tobacco use and genetic disorders as exclusion criteria. This study is part of a research project approved by Tor Vergata.

Technique of Breast Reconstruction With Fat Graft Mixed With PRP

The area destined to receive the graft was determined based on the necessary corrections. Based on this, the harvested material was implanted for breast augmentation mainly into 3 areas: inferior breast rim, superior and inferior region of the areola, and in the superior lateral quadrant.

After pretunneling, fat tissue was implanted (280 mL average; range 80 mL/400 mL, 120 mL average for each breast) at different levels using a delivery cannula (1-2 mm in diameter) with precise, controlled movements. Several layers were laid down to increase the contact surface between the receiving tissue and the implant; this technique is of fundamental importance to allow each layer deposited to survive by diffusion during the few days necessary for growth of blood vessels, which will nourish the implant permanently.^{1,2,5,6} The incisions were closed with 5-0 nylon sutures, and no compressive bandage was applied.

PRP Preparation

The current systems for preparation of platelet concentrations routinely report the use of various centrifugation rates (we used a 1100g centrifuge). After centrifugation, the buffy coat layer, consisting of platelets and white blood cells, was sequestered in a volume of 9 cc of plasma.

In our procedure, PRP was prepared in the presence of a transfusional service doctor, from a small volume of blood (18 cc) according to the Cascade system method,¹⁷ a commercially approved formulation. Briefly, to prepare PRP, blood was taken from a peripheral vein using sodium citrate as an anticoagulant. The systems for preparing

platelet concentrations use 1100g for 10 minutes. The PRP protocol uses Ca^{2+} to induce platelet activation and exocytosis of the alpha granules. We added Ca^{2+} when the fat was centrifuged.

The final aim was to obtain a platelet pellet, although the preparation is not selective and includes leukocytes. The secretion of growth factor begins with platelet activation.

Fat Graft Centrifugation According to the Coleman Procedure and Mixing With PRP

Before proceeding to activation of PRP, under general anesthesia, we harvested fat tissue from the abdominal region using some specific cannula. Maintaining asepsis, we took the plunger from the syringes and after closing them with a cap, we positioned them to lie flat in the sterile centrifuge. The syringes were processed for 3 minutes at 3000 rpm/min. This procedure obtains purified fat tissue, preserving the integrity of the adipocytes but separating the fluid fat portion from the serous-bloody part. We mixed 0.5 mL of PRP with 1 mL of centrifuged fat tissue. The purified body fat mixed with PRP was put in 1-mL syringes and aseptically reinserted using the specific microcannula for implanting.

Clinical Evaluation Method

Two methods for the clinical evaluation of outcomes were used: (1) team evaluation and (2) patient self-evaluation. The team evaluation is an evaluation method based on clinical observation, using a scale of 6 values (excellent, good, discreet enough, poor, and inadequate). The patient-based self-evaluation uses the same 6 values mentioned above. The factors/variables that were taken into account were pigmentation, vascularization, pliability, thickness, itching, and pain.

The percentage of maintenance restored was clinically evaluated using 2 different criteria. The first was subjective evaluation and the second, objective evaluation. The subjective evaluation was based on the personal score of each patient, focused on the following parameters: (1) presence of asymmetry, deformity, irregularity, dyschromia, dysesthesia, paraesthesia, and pain; (2) results of the superoexternal quadrant, inferoexternal quadrant, superointernal quadrant, and inferointernal quadrant; (3) reabsorption of fat in one or more regions; (4) time of stabilization of the transplanted fat; and (5) need for retreatment.

For each parameter, patients gave a yes/no or positive/negative evaluation, and percentage of maintenance restored was calculated as the mean of all calculated single percentages. The objective evaluation was made based on the analysis of the preoperative and postoperative photos. The photos were of the same size, brightness, and even contrast. According to the parameters reported above, the operator similarly calculated the percentage of

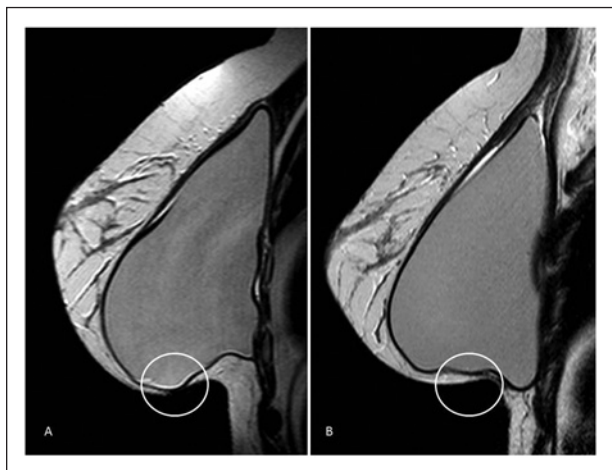


Figure 1. A. Preoperative view: volumetric defect in hypoplastic breast. B. Postoperative view: patient treated with breast prosthesis and implantation of adipose tissue mixed with PRP to restore the volume defect and give a coverage to the implant

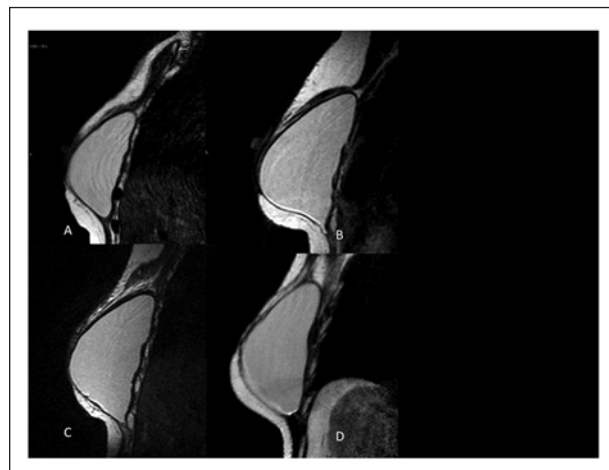


Figure 3. A. Preoperative view: patient with bilateral outcome of capsular contracture and replacement of the implants. B. Postoperative view: improvement of breast contour after 1 treatment and 1 year. C. After 2 treatments and 2 years. D. After 3 years and 4 treatments

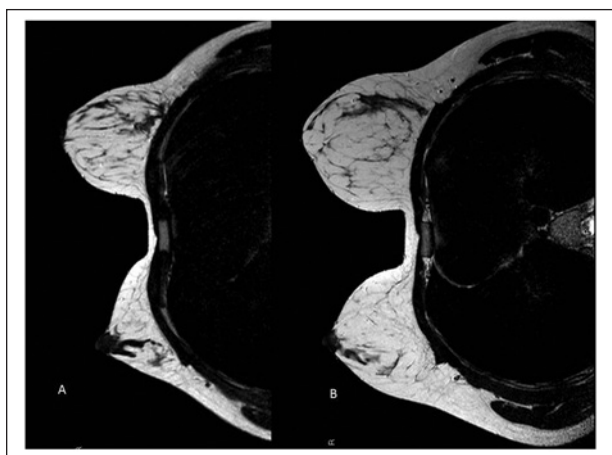


Figure 2. A. Preoperative view: patient affected by monolateral hypoplastic breast defect. B. Postoperative view: after 2 operating sessions, there is great improvement in the volume without use of a breast implant

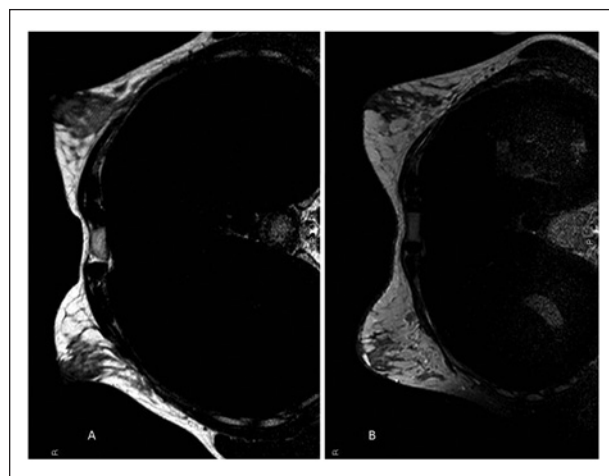


Figure 4. A. Preoperative view: bilateral volume defect. B. Postoperative view: improvement of breast volume

restored material. Finally, the mean of patient and operator evaluations was calculated.

Instrumental Imaging Evaluation Method

The percentage of maintaining restored was evaluated using MRI (Figures 1-4) at the following time points: preoperative, after 6 months, 12 months, and then annually.

MRI showed that transplanted fat tissue survived and formed a significant thickness of the fatty layer (Figures 1B, 2B, and 4B) not only subcutaneously on and around the mammary glands but also between the mammary glands and the pectoralis muscles.

Although small cystic formation and macrocalcification were detected in 1 case, the macrocalcification was easily distinguished from that associated with breast cancer, and the overall cosmetic results were generally satisfactory and encouraging. Almost all the patients were satisfied with their enlarged and soft breasts with a natural contour (Figures 3B, 3C, and 3D).

Statistical Analysis

Values as mean plus standard error were analyzed by means of Student's *t* test, and differences were considered statistically significant for $P < .05$. For 3 or more

groups of univariate data, single-factor ANOVA was used to obtain *P* values.

Discussion

In this case series, supplementation of autologous fat grafts with PRP improved breast soft-tissue defects, compared with centrifuged fat grafting alone. Although small cystic formation and microcalcification were detected in 1 case, the microcalcification was easily distinguished from that associated with breast cancer by an ultrasound scan, and the overall cosmetic results were generally satisfactory and encouraging.

The lipofilling procedure can modify radiological images; however, this interference has been studied in the literature,²⁰⁻²² and radiological studies suggest that imaging technologies (ultrasound, mammography, and MRI) can identify the microcalcifications caused by fat injection.²³ Moreover, recent follow-up studies demonstrated the safety of the procedure, detecting no increase in new disease or tumor recurrence.^{3,20,24} Almost all the patients were satisfied with their enlarged and soft breasts with a natural contour. MRI showed that transplanted fat tissue survived and formed a significant thickness of the fatty layer not only subcutaneously on and around the mammary glands but also between the mammary glands and the pectoralis muscles. Maximum breast augmentation using the described technique varied among the patients and appeared to be 80 to 150 mL. Although these volumes may be smaller than those achieved with large artificial implants, a definite advantage is that patients need not be concerned about postoperative complications induced by artificial implants, such as rupture, infection, capsular contracture, unnatural contour, hardness, neurological symptoms, and immune response. Compared with patients who underwent conventional autologous fat graft to the breasts, augmentation effects were apparently higher with PRP mixed with fat.

For each injection, a 0.5-cm to 1.2-cm increase in breast soft-tissue volume is common with the conventional procedure, compared with the 1.2-cm to 2.0-cm increase seen in this trial of PRP mixed with fat, although the augmentation effect varied among patients. The measurement system we recently devised may help quantify the difference in augmented volume in the future.

Implanted adipose tissue must survive by a simple diffusion mechanism until an active blood supply is reestablished. Thus, survival of the graft, particularly of a larger volume graft, is a balance between this process and hypoxia-induced cell death.²⁵ Prosurvival factors may therefore promote long-term retention and hence durability of the graft. In an animal study, this effect was achieved by using gene therapy to deliver vascular endothelial

growth factor (a potent proangiogenic factor) to the graft. This resulted in increased blood vessel density within the graft and a significant improvement in graft retention at 15 weeks.²⁶

The traditional preparation of growth factors contained in PRP consists of a slow centrifugation, which allows the platelets to remain suspended in the plasma, whereas the leukocytes and erythrocytes are displaced to the bottom of the tube. The final aim was to obtain a platelet pellet, although the preparation is not selective and includes leukocytes. The secretion of growth factor begins with platelet activation. The PRP protocol uses Ca^{2+} to induce platelet activation and exocytosis of the alpha granules.

Calcium acts as a necessary cofactor for platelet aggregation.¹⁷ When Ca^{2+} is used to induce platelet activation, the secretion of the growth factors contained in the granules is slow.¹⁷ To optimize the secretion process, the optimum concentration of Ca^{2+} was previously determined.¹⁷

Standard cell separators and salvage devices can be used to produce PRP. These devices operate on a unit of blood and typically use a continuous-flow centrifuge bowl or continuous-flow disk separation technology and both a hard (fast) and soft (slow) spin, yielding platelet concentrations from 2 to 4 times baseline values.^{27,28} Such devices include the CATS (Fresenius, Wilmington, DE), Sequestra (Medtronic, Minneapolis, MN), Haemonetics Cell Saver 5 (Haemonetics Corp, Braintree, MA), and others.²⁷⁻²⁹ Many surgical procedures require the use of relatively small volumes of PRP.³⁰ Consequently, small, compact office systems have been developed that produce approximately 6 mL of PRP from 45 to 60 mL of blood.^{30,31} There are many such systems, including the GPS (Biomet, Warsaw, IN), the PCCS (Implant Innovations, Inc, Palm Beach Gardens, FL), the Symphony II (DePuy, Warsaw, IN), the SmartPRP (Harvest Technologies Corp, Norwell, MA), and the Magellan (Medtronic, Minneapolis, MN).^{27,30,31} Although all operate on a small volume of drawn blood (45-60 mL) and on the principle of centrifugation, these systems differ widely in their ability to collect and concentrate platelets, with approximately 30% to 85% of the available platelets collected and from a less than 2-fold to an approximately 8-fold increase in the platelet concentration over baseline.^{27,28}

There are several devices for PRP preparation, such as Fibrinet (Cascade Medical Enterprises, Plymouth, Devonshire, UK), Regen (Regen Lab, En Budron B2, CH-1052 Le Mont-sur-Lausanne, Switzerland), Plateltex (Plateltex SRO, Bratislava, Slovakia), and Vivostat (Vivostat A/S, Borupvang 2, DK-3450 Allerød, Denmark).

In general, most systems, whether large or small volume, do not concentrate the plasma proteins of

the coagulation cascade.^{27,29} The concentration of plasma protein levels above baseline can be achieved through secondary ultrafiltration, as is done with the UltraConcentrator (Interpore Cross, Irvine, CA) and the Access System (Interpore Cross), in which the buffy coat collected from a centrifugation stage is passed through hollow fibers with an effective pore size of 30 kDa. This system removes by filtration up to two thirds of the aqueous phase; thus, the concentrations of the retained plasma proteins and formed elements are increased substantially.³²

Anitua et al³³ reported the use of 2 centrifugation rates; the blood was collected into 3.8% (wt/vol) sodium citrate and centrifuged at 4500g for 12 minutes at 4°C to obtain platelet-poor plasma or at 460g for 8 minutes to obtain PR plasma. Calcium chloride was added to platelet-poor and PR plasma at a final concentration of 22.8 mM. The secretion of growth factor begins with platelet activation.

In an interesting study, Mazzucco et al³⁴ described the different growth factor concentrations that are obtained through the different devices (Fibrinet, Plateltex, and Regen) and the homemade method. The platelet-derived growth factor-BB, transforming growth factor (TGF)- β , and insulin-like growth factor (IGF)-1 were detected in lower concentrations with the use of Fibrinet. In contrast, the Regen yielded high concentrations of TGF- β , b-fibroblast growth factor, and IGF-1, whereas the Plateltex yielded high levels of epidermal growth factor.³⁴

A variety of new innovations, including stem cell technology and the stromal vascular fraction, may be developed and may contribute to the improvement of autologous tissue transplantation and regeneration. Further improvements of the technique may cause autologous tissue transfer to become the first choice for breast augmentation in the future.³⁵⁻³⁸

Furthermore, it should be noted that there are other types of scaffolds for breast reconstruction. Colwell et al³⁹ in a retrospective review of 331 consecutive breast reconstructions with acellular dermal matrix demonstrated that this matrix offers a cost-effective reconstruction with a low complication rate. In addition, a recent review showed that acellular dermal matrix in 2-stage expander/implant reconstruction offers a safety profile similar to that of standard submuscular techniques.⁴⁰ Several studies have been published on acellular matrix use, showing both the advantages and disadvantages of this technique.^{41,42} We believe that this matrix may be the procedure of choice in select patients.

Results

We observed a 69% maintenance of contour restoring and 3-dimensional volume after 1 year ($P < .0001$ vs control group) in the patients treated with reconstructive

3-dimensional projection of breast by fat grafting and PRP. Transplanted fat tissue resorption was analyzed with instrumental imaging, nuclear MRI (as suggested Salgarello et al⁴³), and ultrasound. Compared with breast reconstruction using implants of the same size, augmentation with PRP mixed with fat tissue resulted in a lower height but a more natural contour and softness of the breasts. All the patients were satisfied with the resulting texture, softness, and contour, and MRI confirmed maintenance of the restoration.

As reported, PRP mixed with fat grafting demonstrates an improvement in maintaining breast volume in patients affected by breast soft-tissue defects.

Conclusion

Breast reconstruction with adipose tissue mixed with the PRP can restore volume satisfactorily. Moreover, this technique can improve skin quality in cases of scarring or radiotherapy outcomes. In some cases, it is possible to give coverage to breast implants and restore residual volume defects. In cases of breast hypoplasia, we can restore the volume, in repeated treatments, to obtain a natural result, with no visible scars. In particular, this is an important advantage in young patients.

In our preliminary study, satisfactory clinical results were generally achieved without any major complications. Thus, we can conclude that PRP mixed with fat graft is sufficiently safe for continuation of the study, though controlled investigations and accumulated long-term results are needed to elucidate the overall safety and efficacy of the treatment. Moreover, additional studies are necessary to further evaluate the efficacy of this method.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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