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ORIGINAL ARTICLE

Autologous Platelet-Rich Plasma Mixed with Purified Fat Graft in Aesthetic Plastic Surgery

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Abstract Platelet-rich plasma (PRP) is a platelet concentrate that has widely been used to accelerate the healing of soft and hard tissues. The preparation of PRP has been described by several authors. Preparation protocols vary from system to system, depending on the concentration of different integrating proteins. The objective of this article is to describe the principal use of PRP mixed with fat grafts in aesthetic plastic surgery.

Keywords Growth factors · Platelets · Platelet-rich plasma · Lipostructure

Platelet-rich plasma (PRP) is a platelet concentrate that has been used widely to accelerate soft-tissue and hard-tissue healing [1]. The preparation of PRP has been described by several authors [2–4]. Preparation protocols vary from system to system, depending on the concentration of the different integrating proteins [5]. The objective of this article is to describe the major effects of PRP mixed with purified and centrifuged fat grafts in aesthetic plastic surgery. The authors treated patients who were affected by deficits of soft tissue with loss of volume and elasticity with PRP mixed with centrifuged fat tissue. PRP derives by methods of autologous platelets concentration which is added to surgical wounds or grafts and to other injuries that require supported or accelerated healing [6].

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Materials and Methods

The authors treated 15 patients (11 female and 4 male) for soft-tissue deficits with loss of volume and elasticity and signs of aging with PRP mixed with centrifuged fat tissue. Patient ages ranged between 20 and 65 years. The patients were treated with the Coleman technique called "lipostructure" [7, 8]. The preoperative study included a complete clinical examination, a photographic examination, and magnetic resonance (RM) of the soft tissue. In addition, in the more complex cases, a high-resolution CT scan with three-dimensional imaging for a better view of the anatomical structures was performed. Postoperative follow-up took place after 2 and 5 weeks, 3, 6, and 12 months, and then annually.

Platelet-Rich Plasma and Growth Factors

Numerous proteins are contained within the α granules of platelets that increase the healing process. These proteins include transforming growth factor (TGF)-β (including β_1 and β_2 isomers), platelet factor 4 (PF4), interleukin (IL)-1, platelet-derived angiogenesis factor (PDAF), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), platelet-derived endothelial growth factor (PDEGF), epithelial cell growth factor (ECGF), insulin-like growth factor (IGF), osteocalcin, osteonectin, fibrinogen, vitronectin, fibronectin, and thrombospondin (TSP) [2, 5]. Collectively, these proteins are members of the families of growth factors, cytokines, and chemokines which, for the purpose of this review, are broadly referred to as secretory

proteins. The many proteins secreted by the activated platelets influence many aspects of wound healing; Anitua et al. [9, 10] have provided a recent, detailed review. Some of the secretory proteins released from platelets are absent in chronic, nonhealing wounds, providing further evidence of their role in wound healing [11, 12].

Patients

Case 1

A 46-year-old female presented with a facial deficit of soft tissue. A clinical objective examination showed loss of elasticity and volume in the temporal, zygomatic, orbital, buccal, and mandibular region (Figs. 1, 2, 3 and 4).

In the presence of a transfusional doctor, PRP is prepared with a small volume of blood (18 ml) taken from a peripheral vein. In fact, we observed a law that considered platelet-rich plasma like blood-component in autotransfusional regime. The preparation of PRP with the

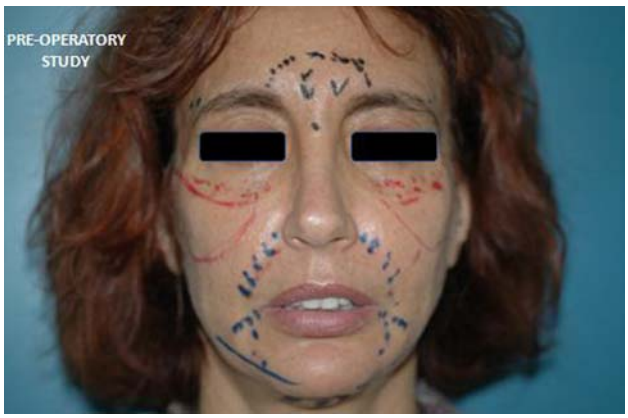

Fig. 1 Case 1 preoperative view with areas to be treated marked

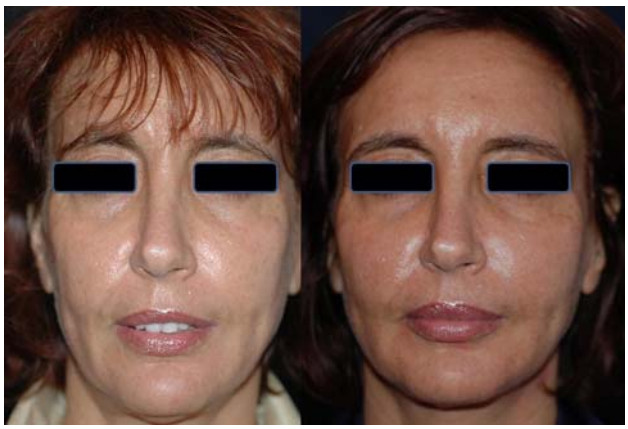

Fig. 2 Case 1 *left* preoperative view. *Right* postoperative view after augmentation of soft tissue in zygomatic and cheek areas after 9 months

Fig. 3 Case 1 *left* preoperative oblique view. *Right* postoperative oblique view

Fig. 4 Case 1 *left* preoperative lateral view. *Right* postoperative lateral view

Cascade-Esforax System consists of centrifugation at 1100 *g* for 10 min. The secretion of growth factor begins with platelet activation. The Cascade-Esforax protocol uses Ca^{2+} to induce platelet activation and exocytosis of the α granules. Calcium acts as an essential cofactor for platelet aggregation. We obtained 9 ml (average) of PRP and used 0.5-0.3 ml (average) of platelet gel actively mixed with 1 ml of centrifuged tissue, favoring tissue growth by new synthesis of collagen. Platelet gel must be added to the fat tissue within 4-5 min after activation with CaCl. When the PRP is in liquid form, the authors can easily add to the PRP to the adipose tissue, because the spill with a 10 ml syringe with needle into the Luer-Look syringe containing centrifuged fat tissue, and mixed. Conversely, when the PRP is in solid form, the authors find difficulty in the spill into the syringe containing centrifuged fat, because the plunger of the syringe containing the PRP is hampered by the strong consistency of the PRP that prevents leakage through the needle.

The abdominal region was injected with solution based on 1 fl of adrenalin in 500 cc of cold physiological solution and 3 fl of Naropin™ 7.5 mg/ml. After 5 min we harvested

180 ml of fat (in general, a variable amount of fat is harvested depending on the size of the defect to be corrected, usually 60-120-180 ml of adipose tissue) from the abdominal region (or in other cases from the lateral portion and/or the medial thighs, knees, hips), using cannulas 3.0-4.0 mm in diameter. Maintaining asepsis we removed the plunger of syringes (10 ml), closed the syringes with their caps, and laid them flat in the sterile centrifuge. The syringes were processed for 3 min at 3000 rpm/min. This procedure yields highly purified fat tissue, preserving the integrity of the walls of the adipocytes but separating the fluid fat portion from the serous bloody part.

In addition, we added 0.3–0.5 ml of liquid PRP (which was previously obtained) in the 10-ml Luer-Look syringe containing centrifuged adipose tissue. These syringes are positioned in the centrifuge vertically without the plungers to facilitate the addition of liquid PRP. After this procedure, the PRP is mixed with the centrifuged fat in the 10-ml Luer-Look syringes and transferred into 1-ml Luer-Look syringes. The PRP mixed with fat tissue is aseptically reinserted using specific microcannulas for implanting (1.5-mm diameter). The location on the face that was to receive the implant was selected by an accurate study of the needed corrections (Fig. 1). The harvested material was implanted in the selected areas in the following quantities:

- 13 ml in the right zygomatic region; 13 ml in the left zygomatic region
- 21 ml in right cheek; 21 ml in left cheek
- 2 ml glabellar area
- 4 ml in the right temporal area; 4 ml in the left temporal area
- 3 ml in the right lower orbital area; 3 ml in the left lower orbital area
- 4 ml in the right nasolabial fold; 4 ml in the left nasolabial fold

We closed the access incisions with 6-0 nylon stitches and applied a compressive bandage. We obtained the desired thickening of the skin but we did not achieve the contour of the face that is obtainable only through well-represented subcutaneous tissue.

Case 2

A 53-year-old female presented with signs of facial aging. The clinical objective examination showed facial asymmetry and loss of elasticity in the zygomatic, orbital, cheek, buccal, and mandibular regions, and lower-eyelid ptosis (Figs. 5 and 6). PRP mixed with fat tissue was used in subcutaneous tissue for the reconstruction of deficient areas.

The abdominal region was injected with a solution based on 1 fl of adrenalin in 500 cc of cold physiological solution and 3 fl of naropyn 7.5 mg/ml. After 5 min we harvested

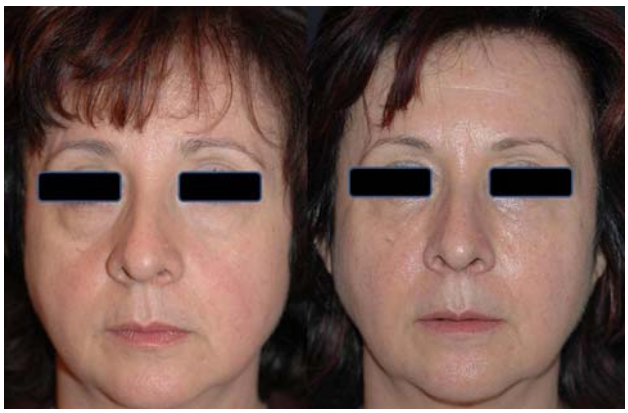

Fig. 5 Case 2 *left* preoperative view. *Right* postoperative view after 11 months

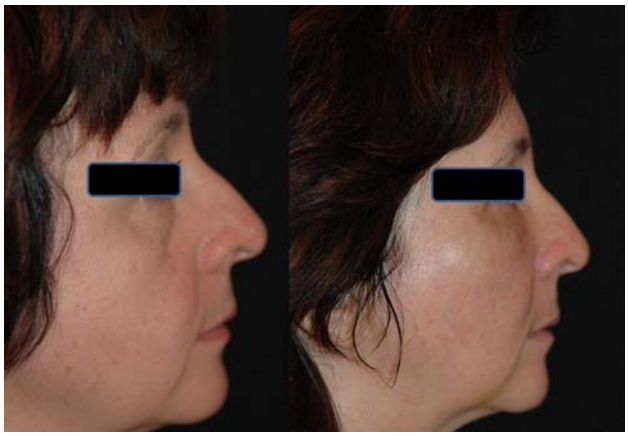

Fig. 6 Case 2 *left* preoperative view. *Right* postoperative view

120 ml of fat from the abdominal region. The harvested material, mixed with PRP, was implanted in the selected areas in the following quantities:

- 12 ml in the right zygomatic region; 12 ml in the right zygomatic region
- 18 ml in the right cheek; 18 ml in the left cheek
- 3 ml in the right nasolabial fold; 3 ml in the left nasolabial fold
- 2 ml in the right lower orbital area; 2 ml in the left lower orbital area

Case 3

A 21-year-old female presented without signs of facial aging and deficit of soft tissue. The objective was augmentation of the zygomatic, cheek, buccal, and mandibular regions (Figs. 7, 8 and 9). PRP mixed with fat tissue was used.

The abdominal region was injected with solution based on 1 fl of adrenalin in 500 cc of cold physiological solution

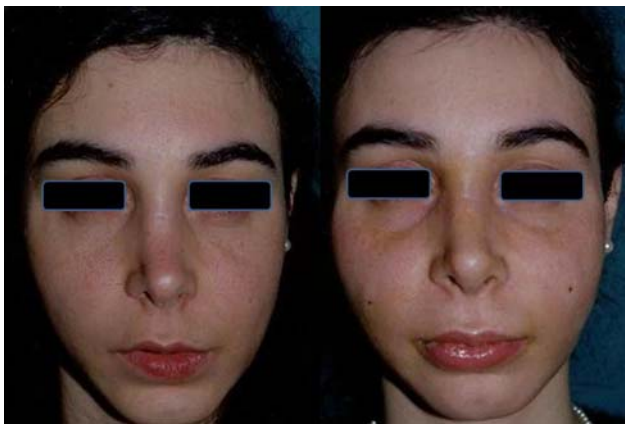

Fig. 7 Case 3 *left* preoperative view. *Right* postoperative view after 15 months

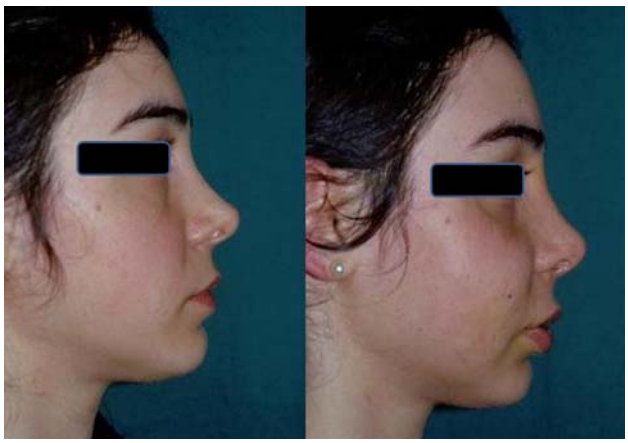

Fig. 8 Case 3 *left* preoperative lateral view. *Right* postoperative lateral view

and 3 fl of naropyn 7.5 mg/ml. After 5 min we harvested 120 ml of fat from the lateral and medial portions of the thighs. The harvested material, mixed with PRP, was implanted in the selected areas in the following quantities:

- 16 ml in the right zygomatic region; 16 ml in the left zygomatic region
- 22 ml in the right cheek; 22 ml in the left cheek
- 5 ml in the buccal rime
- 4 ml in the right nasolabial fold; 4 ml in the left nasolabial fold

Discussion

We feel that there are new issues about the selection of the most appropriate face rejuvenation methods. There are many publications regarding the use of fat grafts in plastic surgery: Guerrerosantos et al. [13] reported the use of fat tissue in patients affected by Romberg syndrome disease

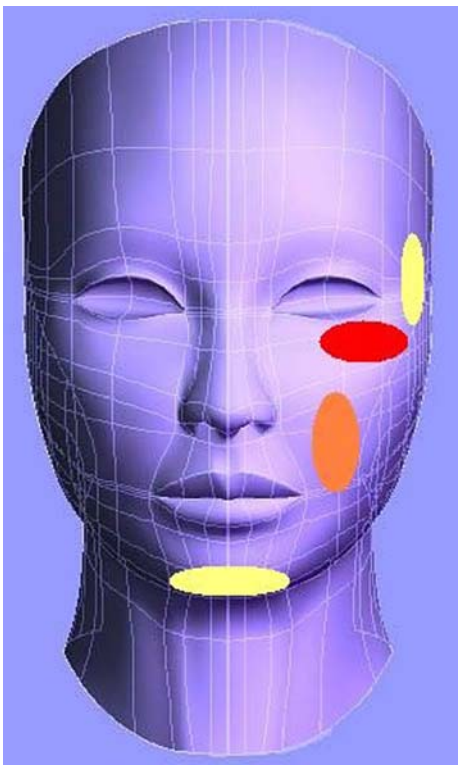

Fig. 9 3D facial study

and facial defects. Based on the facial tissue depression, they separated their cases into four types: For types 1 and 2 they used only fat grafts; for types 3 and 4, according to the case, they used a combined procedure of cartilage and bone graft, free dermis fat graft, and galeal flaps. In addition, they recently reported interesting cases treated with rhytidoplasty combined with pursing plication suspension sutures and lipoinjection. This combination of procedures provides a three-dimensional aesthetic improvement in contour and volume, has a short convalescence and recovery time, and offers less risk for complications, especially in the facial nerve [14].

Nowadays, the lipostructure technique is an alternative to the mid-face lift, augmentation malarplasty, or augmentation genioplasty with prostheses. Lipostructure evolved from lipofilling and is better known as Coleman's technique [15]. It yields not only an increase in volume by utilizing autologous fat, but it also rectifies scars of any kind, including surgical ones. Fat is harvested using thin cannulas with a specific tip designed not to damage adipocytes. An aseptic environment is mandatory to guarantee results. The cannulas are connected to a syringe whose negative intake is controlled by the surgeon to obtain a vital harvest, ideal for transplantation. According to Coleman [15, 16] and the literature, after harvesting, the fat is processed in a centrifuge at 3000 rpm/min for 3 min. The obtained fat tissue is highly purified; the adipocytes are

perfectly separated from the oily component but the integrity of membranes is maintained. This result is not obtainable with lipofilling. The harvested fat, purified from the serous bloody compound, is ready to be implanted. In this step, we add the obtained platelet-rich plasma to the fat tissue and the mixed tissue is implanted at different levels in small tunnels, previously created, forcing the cannulas with precise controlled movements. Small quantities of fat cells, one or two at a time, are left in the exiting movement of the cannulas to create a large grid to favor a correct vascular development around each fat cell. Layers of aligned single cells are placed to increase the contact surface between the receiving tissue and the implant. This technique is fundamentally important; it allows each single layer deposited to survive during the few days necessary for the growth of blood vessels which will nourish them permanently [16]. Compared with lipofilling, fat cells are deposited in rows and survival of the implants is more probable due to reduction of fat necrosis due to the improved vascular development in the implanted area.

Platelets isolated from peripheral blood are an autologous source of growth factors. When platelets, in a concentrated form, are added to graft materials, there is a more predictable outcome. Platelet-rich plasma is an easily accessible source of growth factors for supporting bone and soft-tissue healing. The use of PRP in place of recombinant growth factors has several advantages: growth factors obtained from platelets not only have their own specific effect on tissues but also interact with other growth factors, resulting in the activation of gene expression and protein production [3, 17, 18]. Therefore, the properties of PRP are based on the production and the release of multiple growth and differentiation factors upon platelet activation. These factors regulate and stimulate the healing process, and they play an important role in regulating cellular processes such as mitogenesis, chemotaxis, differentiation, and metabolism [4, 19, 20].

In general, platelet concentrates are blood-derived products used for the prevention and treatment of hemorrhages due to serious thrombopenia of the central origin. PRP is an autologous modification of fibrin glue, which has been described and used in various applications with apparent clinical success. PRP obtained from autologous blood is used to deliver growth factors in high concentrations to the site of bone defects or a region requiring augmentation [3]. We described our experience with the use of PRP mixed with fat grafts in patients affected by facial aging characterized by atrophy of the subcutaneous tissue and deficits of soft tissue with loss of volume and elasticity; in the advanced stage the face is very emaciated, skin is very thin and crinkled, and sebaceous glands are atrophic. The most obvious sign is loss of facial fullness and loss of three-dimensional parameters (atrophy of orbital, palpebral, zygomatic, cheek, and masseter muscle).

Conclusions

We used 0.3-0.5 ml (average) of platelet-rich plasma actively mixed with 1 ml of centrifuged fat tissue for reconstructing the three-dimensional projection of the face contour, restoring the superficial density of facial tissues. Objective examinations after 18 months showed excellent aesthetic improvement, with facial tissue depression alleviated and the emotional status of the patients improved. The results we obtained prove the efficacy of combining these two treatments and the satisfaction of the patient confirms the quality of the work.

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