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Gluteal Augmentation Using Fat Grafting Enriched with Stromal Vascular Fraction (E-Svf) and Platelet Rich Lipotransfert (Prl)®

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Abstract

Background: The purpose of this study was to review the authors' experience of autologous fat grafting, evaluating the effects related to the use of enhanced stromal vascular fraction (e-SVF) and fat grafting with platelet-rich plasma (PRP) in the maintenance of fat volume in gluteal augmentation, comparing the results with a control group treated with centrifuged fat graft.

Methods: 39 patients aged 18-69 years affected by gluteal soft tissue defects were analyzed. 19 patients were treated with SVF-enhanced autologous fat grafts, and 20 patients were treated with SVF-enhanced autologous fat grafts mixed with platelet-rich plasma. The patients in the control group (n =10) were treated with centrifuged fat grafting injection according to Coleman's procedure.

Results: The patients treated with SVF-enhanced autologous fat grafts showed a 58% maintenance of the contour restoring and of three-dimensional volume after 1 year compared with the patients of the control group treated with centrifuged fat graft, who showed a 37% maintenance. In the patients treated with SVF-enhanced autologous fat grafts mixed with PRP, we observed a 61% maintenance of contour restoring and of three-dimensional volume after 1 year.

Conclusion: As reported, the use of either e-SVF or PRP mixed with fat grafting produced an improvement in maintenance of gluteal volume in patients affected by gluteal soft tissue defect.

Keywords: *Subcutaneous Fat; Adipose Tissue; Subcutaneous.*

Introduction

The areas in which stromal vascular fraction cells have been used include radiotherapy-based tissue damage after mastectomy, breast augmentation, post-mastectomy breast reconstruction, breast implant complications, Calvarial defects, Crohn's fistulas and complex perianal fistula, damaged skeletal muscle, scarring and gluteal soft tissue defect, Pectus excavatus, Dermato fibrosis, Vocal fold augmentation and Parry-Romberg disease and facial lipo-atrophy [1].

Visceral and subcutaneous adipose tissue has been demonstrated to contain progenitor cells able to differentiate in multiple cell lineages [2, 3]. After centrifugation of collagenase-digested adult adipose tissue, a heterogeneous cell population named stromal-vascular fraction (SVF) is obtained [3, 4]. This population contains adult stem cells named adipose-derived stromal cells (ASCs) [4]. ASCs might improve tissue outcomes by increasing vascularity and through the secretion of growth factors that improve tissue survival.

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Recently, the authors published works on the use of fat grafting in the lipostructure technique as described by Coleman [5] mixed with platelet-rich plasma (PRP) in plastic surgery [6], in lower chronic extremity ulcers [7], in hemi-facial atrophy and in breast augmentation [8, 9]. Now, we present our experience using engineered fat grafting with the SVF-enhanced or PRP in gluteal augmentation.

Methods

Patients

39 patients (4 males and 35 females), aged from 18 to 69 years, were treated from January 2008 to September 2017. The patients were divided into two groups:

Group A: composed by 19 patients affected by gluteal soft-tissue defects with loss of volume and elasticity, associated to signs of aging (1 male and 18 females), treated with enhanced stromal vascular fraction (e-SVF) autologous fat graft.

Group B: composed by 20 patients (2 males and 18 females) affected by gluteal soft-tissue defects, treated with SVF-enhanced autologous fat grafts mixed with PRP also named Platelet Rich Lipo transfert (PRL)®.

The authors compared their results with a control group comprised 10 patients aged 18–61 years, all affected by gluteal soft tissue

defects, treated with centrifuged fat grafting injection according to the Coleman procedure.

Exclusion Criteria

The authors considered exclusion criteria. Exclusion criteria were divided into two types: local and general.

General exclusion criteria were: platelet disorders, thrombocytopenia, anti-aggregating therapy, bone marrow aplasia, uncompensated diabetes, sepsis and cancer.

Local exclusion criteria were: infection, cancer, loss of substance.

Clinical evaluation methods

Tissue regeneration and fat graft maintenance was evaluated by the analytical comparison of pre and post-operative images. In addition two methods for the evaluation of outcomes were used: 1. Equipe evaluation, 2. Patient self-evaluation.

The Equipe evaluation is an evaluation method based on clinical observation, using a scale of six values (excellent, good, discreet enough, poor, inadequate). The factors/variables, considered were pigmentation, vascularization, pliability, thickness, itching and pain.

The Patient-based self-evaluation an evaluation method based on clinical observation, using a same scale of six values.

Follow-up the patients were performed at the second and fifth weeks and at 3, 6, 12 months, and then annually.

Platelet Rich Plasma Preparation

Generally, the authors prepared PRP from a small volume of blood (9mL-36 mL) according to the Cascade [10] method, i-STEM-PRP-Kit (BIOSTEMS CO., LTD), or from a large volume of blood (55mL) according to Biomed Device (C-punt; Biomed Device, Modena, Itlay, http://www. biomeddevice.it) and in all cases with the approval of Trasfusional Service.

Briefly, to prepare PRP, blood was taken from a peripheral vein using sodium citrate as an anticoagulant. The current systems for preparing platelet concentrations use various centrifuges. The final aim was to obtain a platelet pellet, although the preparation is not selective and includes leukocytes.

In the i-STEM-PRP-Kit (BIOSTEMS CO., LTD) and Cascade-Selphyl procedure, PRP was obtained from 9mL-36mL of blood (small volume) collected in sterile tubes, 9mL each Cascade and 18/20mL each i-STEM, centrifuged for 10 minutes at 3000 revolutions per minute (RPM) in Cascade procedure, and for 2600RPM x 6minutes in the first spin and 2000RPM for 2minutes for the second spin in the i-STEM proicedure. Following centrifugation, Ca^{2+} was added in Cascade procedure. 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.

To optimize the secretion process, the optimum concentration of Ca^{2+} and Calcium Gluconate was previously determined [10, 11].

In the Biomed Device C-Punt procedure, the Blood (55ml) was subjected to centrifugation cycles at 1200rpm per 10minutes, after which 30ml of the suspension containing Platelet poor plasma (PPP) and Platelet rich Plasma (PRP) was extracted from the centrifuge and positioned in the laser light selector. At the end of procedure the authors obtained 18ml of PRP.

Platelet Rich Lipotransfert Preparation (centrifugation and filtration).

Platelet rich lipotransfert preparation was based on the combined use of stromal vascular fraction cells and PRP. The e-SVF was obtained by the centrifugation and filtration of the autologous fat, according to Fastkit procedure (Fastkit CORIOS Soc. Coop, San Giuliano Milanese, Italy) the European Rules and EMA-CAT suggestions.

Fat harvesting was performed in the same moment of the PRP preparation. The Fat (80ml) was subjected to automatic filtration and centrifugation cycles at 1300rpm per 10 minutes, after which 40ml of the suspension was extracted from the bag. The suspension was further filtered through 120μm filter, and 20ml of the e-SVF suspension was obtained. Subsequently, the e-SVF suspension was added and mixed with the centrifuged fat graft.

Using specific micro-cannulas for implantation, the SVF-enhanced fat graft was added to PRP and transferred into 1-ml syringes and aseptically re-injected into the soft-tissue defect. The authors added 0.2ml of PRP and 0.2ml of SVF to each ml of centrifuged fat graft.

This purified body fat combined with PRP was put in 1-ml syringes and aseptically re-injected [10] to implant it into the area to be treated. Skin incisions about 2 mm in diameter to permit passage of the cannula were made using a no. 11 scalpel blade. The implant location destined to receive the implant was selected by an accurate study of the necessary corrections [11, 12]. Fat tissue combined with PRP was implanted "gently" at different levels in small tunnels around the margins created earlier by forcing the cannula with precise controlled movements. A small quantity of fat cells was laid, one or two at a time, during the exiting movement of the cannula to create a large grid to correct the vascular development around each fat cell.

This technique was of fundamental importance in allowing each single layer deposited to survive through the few days necessary for the growth of the blood vessels that would nourish them permanently [13]. We closed the access incisions with 5–0 nylon stitches, and a compressive bandage was not applied.

Preparation of the SVF-Enhanced Autologous Fat Graft (enzymatic digestion)

The cell and tissue preparation procedure mainly exhibited two phases. Phase 1 started with a syringe liposuction (2706 ml average in all patients; range, 200–6.300 ml) in the abdominal region using 3-mm cannulas. While aseptic technique was maintained, the plunger of the 60-ml syringe was removed, and the tip was closed with a cap. Half of the lipo aspirate (386.57 ml average) was placed into the tissue collection container of the Celution 800/CRS System (Cytori Therapeutics Inc., San Diego, http://www.cytoritx.com). Blood and free lipid was removed from the tissue through a wash cycle, and the Celase 835/CRS Reagent (Cytori Therapeutics) was added to enzymatically digest the tissue, which released SVF. After additional wash and centrifugation cycles, 4-5 ml of the SVF suspension was extracted from the system. In the second phase, the remaining part of the lipo aspirate was added to the tissue collection container and a washing step was automatically carried out. Once completed, the 4-5 ml of SVF suspension was added and mixed with the washed fat graft resulting in approximately 434.5ml (range, 140-750 ml) (217.25 per each side) of SVF-enhanced fat tissue for grafting. This newly proce-

ssed cell-enhanced fat graft typically consists of 25%-30% water, which will be reabsorbed by the body in the postoperative period. This overall process was controlled through automated sensors and processing algorithms that ensured standard handling of the tissue and cells, and the process was completed within 160 minutes. The SVF-enhanced fat graft was transferred into 10ml syringes and aseptically re-injected into the patient using specific micro-cannula for implantation.

The donor site region was infiltrated with a cold saline solution containing 1 ml of adrenaline per 500 ml of saline solution without lidocaine or carbocaine. Adipose tissue was removed after 5 minutes using a 3mm diameter cannula and a 60-ml Toomey syringe. The SVF-enhanced adipose tissue was injected using specific micro-cannula (1-2mm diameter) for implantation.

Results

In Vivo

Influence of platelet-rich plasma in tissue regeneration and during fat grafting surgical procedures Previously the authors published [10] their experience about the application of PRP in Plastic Surgery. The authors added 0.2ml of PRP per each ml of fat tissue.

The first clinical case [Figure 1] corresponds to a female patient affected by buttocks atrophy, age 38, weight 60 Kg, height 168 cm, BMI 21,3, no smoking patient, treated with a contextual liposuction of hips, abdomen, sacred, inner thighs, knees areas and lipofilling of the buttocks.

In detail the authors use a fat graft centrifuged mixed with SVF and PRP (PRL)® and injected in left buttock 250ml and 210ml in right buttock.

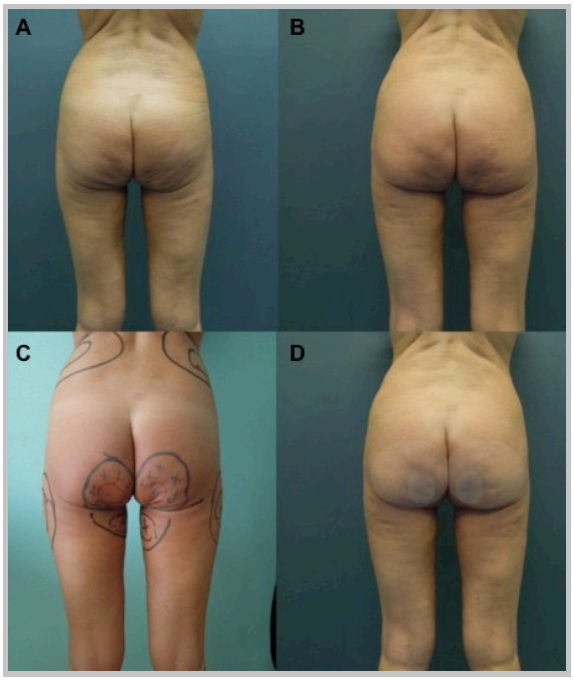

Figure 1: Buttocks atrophy treated with PRL. A) Pre-operative situation of a female patient affected by buttocks atrophy. B) Post-operative situation after 24months and one treatment; the authors use a Platelet Rich Lipotransfert and injected in left buttock 250ml and 210ml in right buttock. C) Pre-operative study. D) Treated areas highlighted.

The second clinical case [Figure 2] corresponds to a female patient affected by HIV with buttocks atrophy, age 57, weight 62 Kg, height 179 cm, BMI 19,4, no smoking patient, treated with a contextual liposuction of hips, abdomen, sacred, back and lipofilling of the buttocks.

In detail the authors use e-SVF method and injected in each buttock 650ml.

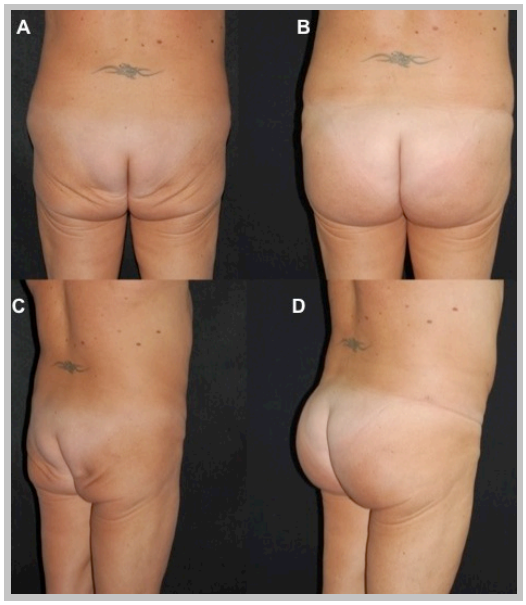

Figure 2: HIV atrophy treated with PRL. A) Pre-operative of a female patient affected by HIV with buttocks atrophy. B) Post operative situation after 14 months and one treatment; the authors use a Platelet Rich Lipotransfert and injected in each buttock 650ml, C) Pre-operative situation in ¾ right projection, D) Post-operative situation in ¾ right projection after 14 months.

The third clinical case [Figure 3] corresponds to a female patient affected by buttocks atrophy, age 53, weight 70 Kg, height 164 cm, BMI 26, no smoking patient, treated with a contextual liposuction of hips, hamp, abdomen, back, groin areas and lipofilling of the buttocks.

In detail the authors use in a first time a fat graft obtained by Celution system and injected for each buttock 160ml and in the second time 350ml for each buttock obtained by normal centrifugation.

The authors observed in patients affected by gluteal soft tissue defect treated with reconstructing three dimensional projection by fat grafting enriched with SVF and PRP at concentration of 0.2ml per each ml of fat tissue, a 61% maintenance of contour restoring and three-dimensional volume after 1 year, and only 37% in control patients (n=10) treated with only fat grafting; when we used e-SVF autologous fat graft we observed a 58% maintenance of contour restoring after 1 year.

Discussion

Starting from the modern concept to minimize complications and skin scars, the use of fat graft seems to embody perfectly the ideal procedure to reshape buttock with modest risks of complications and absence of skin scars. Because of this reason, we prefer avoid using gluteal prosthesis implants, that require skin scars and the risks of complications are considerably higher than fat tissue grafting.

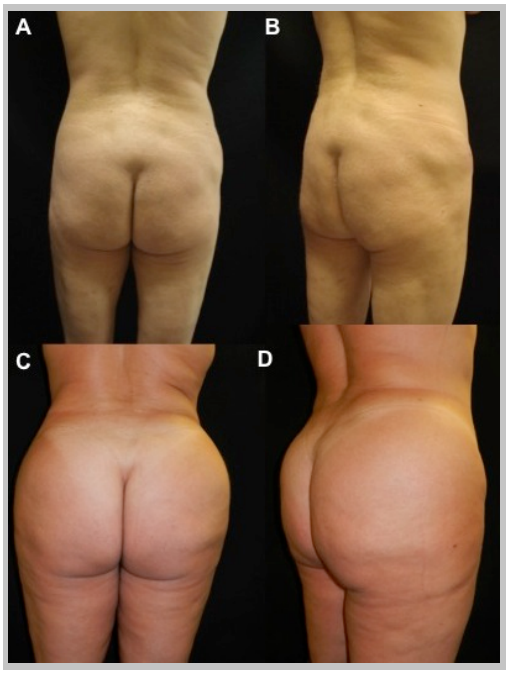

Figure 3: Buttocks atrophy treated with SVFs. A) Pre-operative situation of a female patient affected by buttocks atrophy. B) Pre-operative situation in ¾ right projection. C) Post operative situation in back projection after 20 months and two treatments; the authors use in this first time a fat graft obtained by Celution system and injected for each buttock 160ml; the authors use in the second time a fat graft obtained by Celution system and injected for each buttock 350ml. D) Post operative situation in ¾ right projection after 20 months and two treatments.

The works of authors like Mendieta et al. [14] and Nicareta et al. [15] have introduced lipofilling in gluteal reshaping, that is the center of the rear figure of the body and plays the same role in rear body contour like breast in front body contour.

During the pre-operating study of body contour the buttock can't be dissociate from other areas connected around its circumference. In fact, the buttock reshaping is realized by interaction of liposuction of exceeding areas and lipofilling to restore volumes and fill depressed areas. From the contextual modelling of the hips, the V sacral area, the trochanters and inner thighs, the modelling of buttocks inoculating fat tissue is completed.

According to Mendieta [14] classification, the gluteus may show four different shapes and, depending on the shape, may suggest indication to the procedure. For the purpose of lipofilling, we consider the gluteus such as an hemisphere that has to be recreated.

The anatomical planes in which fat tissue is being injected are the subcutaneous one and the intramuscular one, as well as in case of breast asymmetry, even if in less involved cases the technique is particularly versatile for asymmetries.

In fact the cases we have been treated are both for aesthetic reason and reconstructive reason related to HIV lipo-dystrophy pathology and congenital or acquired asymmetries. This is the only technique that has allowed us to obtain ideal results, very appreciated by patients and with very few complications. The limit of this technique is essentially related to the amount of fat tissue available, to the long-time intra-operative and the costs of the technique.

When 0.2mL of PRP and 0.2mL of SVF was injected mixed with 1 ml of centrifuged fat, it favoured growth and restored fat volume maintenance. PRP, being produced during surgical procedures under sterile conditions, is easy to produce and safe to use; moreover, PRP is lacking of surface antigens, responsible of potential allergic reactions [16].

Our results clearly documented that the use of PRP and SVF during fat grafting favours adipose tissue maintenance and survival. Moreover, our in vitro data are in accordance with the hypothesis that PRP stimulates adipose tissue regeneration, as demonstrated in controlled animal studies for both soft and hard tissues [17]. In addition, comparing to lipofilling [18-20] where fat cells are laid in rows without solution of continuity, implant survival is likely derive from reduction of fat necrosis due to improved neo-angiogenesis in the implanted area.

Now, there are new issues in literature about the selection of the most appropriate PRP methods or SVF isolation. Indeed, there are many publications regarding the use of different kit of PRP preparation based on different speed and modality of centrifugation.

Anitua et al. [21] reported the use of two centrifuges; sample of blood were collected into 3.8% (wt/vol) sodium citrate were centrifuged either at 4500g for 12 min at 4°C to obtain PP-plasma or at 460g for 8 min to obtain PR-plasma. Calcium chloride was added to PP- and PR-plasma of each donor at a final concentration of 22.8 ml [21].

Standard cell separators and salvage devices can be used to produce platelet-rich plasma. These devices operate on a unit of blood and typically use continuous-flow centrifuge bowl or continuous-flow disk separation technology and both a hard (fast) and a soft (slow) spin, yielding platelet concentrations from two to four times baseline [22].

Many surgical procedures require use of relatively small volumes of platelet-rich plasma.

Consequently, small, compact office systems have been developed that produce approximately 6 ml of platelet-rich plasma from 45 to 60 ml of blood [23].

The authors used in the personal experience C-Punt Biomed Device (C-punt; Biomed Device, Modena, Itlay, http://www.biomeddevice.it), Regen (Regen Lab, En Budron B2, CH-1052 Le Mont-sur-Lausanne, Switzerland), Cascade-Fibrinet (Cascade Medical Enterprises, Plymouth, Devonshire, UK), Angel (Cytomedix, Inc. 209 Perry Parkway Suite 7 Gaithersburg, MD 20877-2143), Vivostat (Vivostat A/S, Borupvang 2, DK-3450 alleroed, Denmark), i-STEM-PRP-Kit (BIOSTEMS CO., LTD.)

In general, a lot of systems do not concentrate the plasma proteins of the coagulation cascade [22]. The concentration of plasma proteins 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.

With this system, up to two-thirds of the aqueous phase is removed by filtration; thus, the concentrations of the retained plasma proteins and formed elements are correspondingly increased [18].

About the use of PRP, the authors feel that there are new issues in literature about the selection of the most appropriate regenerative methods. Indeed, there are many publications regarding the use of PRP with/without fat graft in plastic and reconstructive surgery.

Powell et al. [24], describe anti-inflammatory properties with reduced edema and ecchymosis associated with the autologous platelet gel in eight women after deep-plane rhytidectomy (face lifting).

PRP was also shown to be effective in stopping capillary bleeding in the surgical flaps of a series of 20 patients undergoing various cosmetic surgery (face lift, breast size changes or neck lifts) reported by Man D [23].

Guerrerosantos et al. [25] reported the use of fat tissue without PRP in patients affected by Romberg syndrome disease. In addition, they recently reported interesting cases treated with rhytidoplasty combined with pursing plication suspension sutures and lipo-injection [25].

Recently, the authors described the use of fat graft with platelet rich plasma and with only lipostructure technique [13] in patients affected by Romberg Syndrome.

Lipostructure evolved from lipofilling and is better known as Coleman's technique [20].

In addition Yoshimura K et al. [1] describe a new methods and new technologies in the use of fat grafting. In fact, they use a Cell-Assisted-Lipotransfer(CAL) for cosmetic breast augmentation using adipose derived stem/stromal cells. In CAL, autologous adipose derived stem stromal cells(ASCs) are used in combination with lipoi-njection. A stromal vascular fraction(SVF) containing ASCs is freshly isolated from half of the aspirated fat and recombined with the other half. This process converts relatively ASC poor aspirated fat to ASC-rich fat.

A new aim could be the use of SVF isolated from half of fat tissue mixed with platelet rich plasma and recombined with the other half.

Actually the SVF technique allows us to prepare the fat graft to be enriched with a procedure using "pure graft" basics, in order to remove that 30% of the liquid element that has been limited the fat grafting in previous technique. A further evolution allows us to measure the regenerative cells total amount obtained by enzymatic digestion, therefore we can calculate exactly the number of cells obtained in enriched fat grafting.

Currently, for automatic SVF extraction, it is possible to use or not enzymatic digestion. In the first case, Celution system (Cytori Therapeutics, Inc., San Diego, CA, http://www.cytori.com) was used by the authors until to 2014. Cell viability by trypan blue exclusion was consistently more than 98%. The cell yield was ~50,000 ± 6,956 nucleated cells per mL of adipose tissue. In the second case, it is possible to isolate SVFs by mechanical centrifugation and filtration of the fat. Briefly, through the use of modified FASTKIT system (CORIOS Soc. Coop, San Giuliano Milanese, Italy, http:// www.corios.it), the fat (80 mL) was subjected to automatic filtration and centrifugation cycles at 1100g per 10 minutes, after which 40 mL of the suspension was extracted from the bag. The suspension was further filtered through a 120-µm filter, obtaining about 20 ml of SVF suspension. The latter was centrifuged at 600g for 10 min and then pellet was resuspended in erythrocyte lysis buffer and incubated for 5 min at room temperature. After centrifugation at 600g for 5 min, the pellet was resuspended in few microliters of growth medium and cellular population was counted using hemocytometer. Cell viability by trypan blue exclusion was consistently more than 98%. About 65,000 ± 3,345 nucleated cells/mL of fat tissue were obtained.

Our purpose remains to enrich our graft with 50,000-70,000 cells each mL of fat tissue.

This work suggest two fundamental points: first, PRP added in concentration of 0.2ml (20%) per each ml of fat tissue favours an optimal ASCs proliferation with correct architectural adipocytes distribution better cell-to-cell interaction, adipose tissue growth, and

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