

Application of Platelet-Rich Plasma in Plastic Surgery: Clinical and *In Vitro* Evaluation

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The clinical use of platelet-rich plasma (PRP) for a wide variety of application has been reportedly employed most prevalently in problematic wounds, maxillofacial and hemi-facial atrophy, Romberg Syndrome, and diabetic foot ulcers. To our knowledge, PRP has never been described in the enhancement of fat grafting during tissue-engineering application *in vivo*. The authors describe the preparation of PRP and its use in a series of 43 patients who underwent plastic, reconstructive, and maxillofacial surgery for chronic lower extremity ulcers ($n = 18$) and multiple facial applications ($n = 25$). PRP mixed with fat grafting was used in 76% patients affected by multiple facial diseases and in 88.9% patients affected by lower extremity ulcers. PRP injection alone was used in the remaining patients. The authors observed that after a 7.1-week and 9.7-week (average) course of twice-daily wound treatment with PRP suspended on a collagen base, 61.1% and 88.9% of chronic lower extremity ulcers underwent to 100% reepithelization compared with 40% and 60% of controls ($n = 10$) treated with hyaluronic acid and collagen medication. In patients treated with reconstructing three-dimensional projection of face by fat grafting and PRP, we observed a 70% maintenance of contour restoring and three-dimensional volume after 1 year compared to only 31% of controls ($n = 10$) treated with fat grafting alone. *In vitro*, PRP induced a significant increase in the number of adipose-tissue-derived stem cells compared to control cultures. These results documented that PRP accelerates chronic skin ulcer reepithelization and improves maintenance and function of fat graft in patients who underwent plastic reconstructive surgery, possibly by stimulating adipose-tissue-derived stem cell proliferation.

Introduction

PROponents of PLATELET-RICH PLASMA (PRP) technology suggest that its benefits include an increase in hard- and soft-tissue wound healing and a decrease in postoperative infection, pain, and blood loss.¹⁻³ Several publications report the use of PRP for clinical applications, including periodontal^{4,5} and oral surgery,⁶⁻⁹ maxillofacial surgery,^{9,10} aesthetic plastic surgery,¹¹⁻¹³ spinal fusion,¹⁴⁻¹⁶ heart bypass surgery,¹⁷ and treatment of soft-tissue ulcers.^{18,19} The details of the quantity of PRP used and the methods of application are procedure specific. Although the vast majority of these studies have yielded excellent outcomes, most consist of case studies or reports of limited series. As such, wound-healing enhancement by PRP remains largely anecdotal.²⁰

The authors present their experience of 43 cases, including reconstructive surgery of the lower extremity in chronic ulcers, and reconstruction of three-dimensional projection of soft tissues of the face in Romberg Syndrome and hemi-

facial atrophy, of chemical injuries and burns sequelae. The aim is to evaluate the effects of PRP obtained from a small volume of blood mixed with fat tissue in comparison with wounds those treated with hyaluronic acid and collagen and in facial soft tissue defect treated only with fat grafting. As a matter of fact, the new approach in plastic surgery for correction of soft-tissue defects by adult stem cell delivery is a fast-growing field of research. Many studies have demonstrated the plasticity and therapeutic potential of stromal cells isolated from human subcutaneous adipose tissue.²¹ The stromal vascular fraction (SVF) obtained by centrifugation from adipose tissue consists of a heterogeneous cell population, including endothelial cells, smooth muscle cells, pericytes, leukocytes, mast cells, and multipotent adipose-tissue-derived stem cells (ASCs).²²⁻²⁴

The data we report prove the clinical efficacy of the combined treatment with PRP; moreover, patients' satisfaction further confirms the quality of the results. After studying this article, the reader should be able to (1) prepare PRP for fat tissue grafting, (2) apply PRP intraoperatively in

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plastic surgery, and (3) evaluate the effect of PRP on ASC cultures.

Materials and Methods

Patients

Clinical data of patients are presented in Tables 1 and 2. From January 2007, 43 cases were treated with PRP at the Plastic and Reconstructive Surgery Department of the "Tor Vergata" University of Rome. The preoperative study was carried out through a complete clinical examination, a photographic examination, and through RM of the soft tissue and trough biopsy punch 2–4 mm of diameter. In addition, in more complex cases, a high-resolution CT scan with 3D imaging for a better view of the anatomical structures has been performed. Postoperative follow-up took place after 2 and 5 weeks and 3, 6, 12, and 18 months. We used PRP mixed with fat grafting in 76% patients affected by multiple facial diseases and in 88.9% patients affected by lower extremity ulcers. We used only PRP injections in 24% patients affected by facial disease and in 11.1% patients with lower extremity ulcers.

PRP preparation

PRP was prepared from a small volume of blood (18 cc) according to the method of Cascade-Esforax system,^{2,3,6} with modifications. Briefly, to prepare PRP, blood was taken from a peripheral vein using sodium citrate as an anticoagulant. The traditional preparation of PRP consisted of a slow centrifugation, which allows the platelets to remain suspended in the plasma, while the leukocytes and erythrocytes are displaced to the bottom of the tube. A rapid centrifugation can cause mechanical forces and can raise the temperature, thus inducing changes in the ultrastructure of platelets that, in turn, can initiate a partial activation, with a consequent loss of its content.^{2,6}

The current systems for preparing platelet concentrations use various centrifuges (the authors use 1100 g for 10 min; Fig. 1A). 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 (Fig. 1B). The PRP protocol uses Ca^{2+} to induce platelet activation and exocytosis of the α granules (Fig. 1C, D). Calcium acts as a necessary cofactor for platelet aggregation.^{2,6} When Ca^{2+} is used to induce platelet activation, the secretion of the growth factors^{25–27} contained in the granules is slow. To optimize the secretion process, the optimum concentration of Ca^{2+} was previously determined.^{2,3,6}

When a rapid activation and coagulation is required, endogenous thrombin can be used.

Fat grafting preparation

Before proceeding to activation of PRP, under general anesthesia we harvested fat tissue from the abdominal region using some specific cannulas. Maintaining asepsis, we took the plunger of syringes; after closing with cap, we positioned them flatly in the sterile centrifuge. The syringes were processed for 3 min 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. The purified body fat was put in 1 mL syringes and aseptically reinserted using the specific micro-cannulas for implanting. The authors mixed 0.5 mL of PRP with 1 mL of

fat tissue centrifuged. The implanting location identified to receive the implant was selected by an accurate study of the necessary corrections. On this basis, the harvested material was implanted for facial diseases in the zygomatic region, cheek, buccal rime, upper and lower eye-lid, temporal area, and orbital area; for lower extremity ulcers, in the lacking, perilesional area. Fat tissue was implanted at different levels in small tunnels, previously created by forcing the cannulas (1.5 mm diameter) with accurate and controlled movements. Small quantities of fat cells were laid, one to two at a time, in the exiting movement of the cannulas to create a large grid to facilitate a correct vascular development around each fat cell. Layers of aligned single cells were laid to increase the contact surface between the receiving tissue and the implant; this technique is of fundamental importance to allow each single layer deposited to survive during the few days necessary to the growth of blood vessels for nourishment.²⁸ We closed the access incisions with a 5-0 nylon stitches, and no compressive bandage was applied.

ASC isolation and expansion

Liposuction aspirates were washed three times with phosphate-buffered saline (PBS) and suspended in an equal volume of PBS and 0.1% collagenase type I (C130; Sigma-Aldrich, Milan, Italy) prewarmed to 37°C.^{29,30} Adipose tissue was placed in a shaking water bath at 37°C with continuous agitation for 60 min and centrifuged for 10 min at 600 g at room temperature. The supernatant, containing mature adipocytes, was aspirated. The SVF pellet was resuspended in erythrocyte lysis buffer (155 mM NH_4Cl , 10 mM KHCO_3 , and 0.1 mM EDTA) and incubated for 5 min at room temperature.²¹ After centrifugation for 5 min, the pellet was resuspended in few microliters of growth medium and passed through a 100- μm Falcon strainer (Becton and Dickinson, Sunnyvale, CA), and cellular population was counted using hemocytometer. In 12 randomly selected patients, we calculated nucleated SVF cells that were 317,578 + 25,645/mL of lipoaspirate. Then digestion was plated in Dulbecco's Modified Eagle's Medium (DMEM) (Euroclone, Pavia, Italy) supplemented with 10% (v/v) fetal bovine serum (FBS; Euroclone), 2 mM L-glutamine, 100 U/mL penicillin, 100 μg /mL streptomycin, and 0.25 μg /mL Fungizone (Invitrogen, Milan, Italy), at a density of 2500–5000 cell/cm² of surface area. This initial passage of primary cell culture was referred as passage 0 (P0). After 48 h of incubation at 37°C at 5% CO_2 , the cultures were washed with PBS and maintained in stromal medium until they achieved 75–90% confluence.^{29,30} ASCs were passaged by trypsin (0.05%) digestion and plated at a density of 5000 cells/cm² (P1). Medium was changed every 3 days. To demonstrate the differentiation capacity of cultured ASCs, adipogenic and osteogenic differentiation was verified in third-passive confluent cells, according to previously published methods.³¹ Briefly, for adipogenesis, ASCs were cultured in DMEM supplemented with 10% FBS, 100 μM L-ascorbic acid, 1 μM dexamethasone, 0.5 mM 1-methyl-3-isobutylxanthine (IBMX), 100 μM indomethacin, and 10 μg /mL human recombinant insulin (Sigma-Aldrich S.r.l, Milan, Italy). Control was cultured in DMEM plus 10% FBS. Medium was changed every 3 days for 3 weeks, and adipogenesis was assessed by Oil Red O staining (Fig. 2A, B). Osteogenic differentiation was induced in DMEM supplemented with 10%

TABLE 1. DETAILS OF PATIENTS RECEIVING PLATELET-RICH-PLASMA MIXED WITH FAT GRAFTING

<i>Pz</i>	<i>Age</i>	<i>Comorbidity</i>	<i>Pathology</i>	<i>Old treatment</i>	<i>Treatment</i>	<i>Complication</i>
C.E.	48	No	Posttraumatic scar	Skin-Graft; Recell	Lipostructure+PRP	No
Z.C.	22	Cardiological disease	Lower extremity ulcer	N°2 Skin Graft	Lipostructure+PRP	No
C.T.	51	No	Postsurgical scar	Breast augmentation	Lipostructure+PRP	No
Y.F.	29	No	Facial postburn scar	Skin-Expander+ skin graft	Lipostructure+PRP	No
B.G.	17	Congenital foot wrong	Foot ulcer+postsurgical scar	Correction of congenital wrong	Lipostructure+PRP	No
M.S. ^a	53	Romberg disease	Hemifacial atrophy left	N°1 Poliglactac acid, N°1 lipostructure	N°2 Lipostructure+PRP	No
D.M.	37	No	Nose and facial deficit+ posttraumatic scar	Rhinoplasty	Lipostructure+PRP	No
M.P.	39	No	Hemifacial atrophy+ postsurgical scar	Removal angioma	Lipostructure+PRP	No
D.A.	30	Hcv+	Postburn scar	Skin-Expander+ skin graft	Lipostructure+PRP	No
M.Y.	37	No	Postburn scar	Skin-Expander+ skin graft	Lipostructure+PRP	No
A.M.	20	No	Postsurgical scar after lower extremity ulcer treatment	Skin graft	Lipostructure+PRP	No
S.P.	47	No	Postsurgical scar	Prostheses removal after breast augmentation	Lipostructure+PRP	No
S.E.	66	Diabetes	Lower extremity ulcers	N°2 skin grafts	Lipostructure+PRP	No
A.R.	49	No	Posttraumatic scar	Ophthalmological surgery	Lipostructure+PRP	No
P.T.	87	Hypertension, dislipidemy	Lower extremity ulcer	No	Lipostructure+PRP	No
E.P.	32	Nasal polypus, Synus jaw polypus	Mandibular cyst	Polipectomy+cyst removal	Only PRP	No
V.J.	20	No	Nose and facial deficit+ posttraumatic nasal scar, and hemifacial atrophy	No	Lipostructure+PRP	No
P.F.	44	Romberg disease	Hemifacial atrophy right	No	Lipostructure+PRP	No
A.C.	36	No	Postsurgical scar	Cortison infiltration	Lipostructure+PRP	No
B.D.	21	No	Postburn scar	No	Lipostructure+PRP	No
A.M.	21	No	Ulcer posttraumatic	Skin graft	Lipostructure+PRP	No
S.A.	38	No	Scar+loss of substance	Removal scar	Lipostructure+PRP	No
M.G.	54	Neurological disease	Ulcer posttraumatic	Wound complex	Lipostructure+PRP	No
G.E.	67	Arteriopathy lower extremity	Lower extremity ulcers	Wound complex	Only PRP	No
P.A.	48	No	Posttraumatic scar	No	Lipostructure+PRP	No
V.P.	21	No	Mandibular cyst	No	Only PRP	No
M.F.	43	No	Posttraumatic scar	No	Lipostructure+PRP	No
D.S.	33	No	Edentulia	Sub-mandibular gland removal	Only PRP	No
C.V.	51	No	Empty jaw	No	Only PRP	No
C.A.	48	No	Dysodontiasis	No	Only PRP	Infection
I.S.	16	No	Mandibular cyst	No	Only PRP	Infection
L.S.	29	No	Dysodontiasis	No	Only PRP	No
B.V. ^b	79	Cardiological disease	Lower extremity ulcer	Safenectomy	Lipostructure+PRP	No
V.D.	79	No	Ulcer posttraumatic	No	Lipostructure+PRP	No
Z.C.	43	No	Lower extremity ulcer	N°1 lipostructure	Lipostructure+PRP	No
T.E.	74	Diabetes, cardiological disease	Lower extremity ulcer	N°1 lipostructure	Lipostructure+PRP	No
P.P.	84	Diabetes, Neurological disease, Hypertension, dislipidemy	Sacred Ulcer	N°1 Vacuum therapy	Lipostructure+PRP	No

(continued)

TABLE 1. (CONTINUED)

Pz	Age	Comorbidity	Pathology	Old treatment	Treatment	Complication
K.M.	21	No	Chin deficit	No	Lipostructure+PRP	No
G.M.	51	No	Foot ulcer	No	Lipostructure+PRP	No
M.E.	31	Arteriopathy lower extremity	Lower extremity ulcers	Wound complex	Lipostructure+PRP	No
B.S.	27	No	Foot ulcer	No	Lipostructure+PRP	No
R.G.	29	Neurological disease	Lower extremity ulcer	Wound complex	Lipostructure+PRP	No
D.M.	25	Romberg disease	Hemifacial atrophy	No	Lipostructure+PRP	No
M.F.	63	Diabetes, hypertension, dislipidemy	Foot ulcer	Toilette	Wound complex	Infection
M.D.	47	No	Foot ulcer	No	Wound complex	No
G.G.	43	No	Foot ulcer	Toilette	Wound complex	No
D.D.	54	Dislipidemy	Foot ulcer	Toilette	Wound complex	Infection
D.C.	65	Arteriopathy lower extremity	Lower extremity ulcers	Toilette	Wound complex	No
D.R.	43	Diabetes	Lower extremity ulcer	Amputation	Wound complex	No
B.E.	54	Diabetes, cardiological disease	Lower extremity ulcer	Toilette	Wound complex	Infection
B.R.	44	No	Foot ulcer	No	Wound complex	No
A.E.	31	Arteriopathy lower extremity	Foot ulcer	No	Wound complex	No
A.G.	23	Trauma	Foot Ulcer	N°1 vacuum therapy	Wound complex	No

^aClinical case illustrated in Figure 4.

^bClinical case illustrated in Figure 3.

PRP, platelet-rich plasma; Pz, patients.

FBS, 200 μ M L-ascorbic acid, 0.1 μ M dexamethasone, and 10 mM β -glycerol phosphate (Sigma). Control was cultured in DMEM plus 10% FBS. Medium was changed every 3 days for 21 days. To assess mineralization corresponding to osteogenic differentiation, intracellular calcium deposits were stained with von Kossa (Fig. 2C, D). Images were obtained at 200 \times magnification through a digital camera (Dxm1200F; Nikon, New York, New Jersey) connected to a computer using Nikon ACT-1 software.

Assessment of the effect of PRP on ASC proliferation

ASCs at the third passage were seeded at a density of 5000 cells/cm² in 24-well plates and incubated for 24 h in DMEM containing 10% FBS. Medium was then replaced with DMEM containing 0.1% FBS for starvation. After 24 h the medium was changed, for the treatment, with DMEM + 10% FBS (control), and DMEM + 10% FBS + 5% PRP. Medium containing the respective supplements was replaced

every 2 days. After 0, 2, 4, 6, and 8 days of culture, cells were digested with 0.25% trypsin solution and then counted, with trypan-blue exclusion, using hemocytometer. Cell viability by trypan blue exclusion was consistently more than 98%. The reported results were the mean value of triplicate samples. Each assay was performed twice.

Clinical evaluation methods

Graft success was quantified according to the method Foyatier *et al.*,³² with minor modifications. Briefly, the percentage of maintaining restored was evaluated with two different criteria. The first was the subjective evaluation, and the second one is the objective evaluation. The subjective evaluation was based on the personal score of each patient focused on following parameters: (1) presence of asymmetry, deformity, irregularity, dyschromia, dysesthesia, paraesthesia, and pain; (2) results of the front, temple, cheek, cheekbones, basilar board, upper lip, lower chin, nasal-labial fold, nose, and

TABLE 2. DETAILS OF PATIENTS TREATED WITH LIPOSTRUCTURE (CONTROL GROUP)

Pz	Age	Comorbidity	Pathology	Old treatment	Treatment	Complication
C.M.	46	No	Skin depression	No	Lipostructure	No
D.F.	37	No	Facial atrophy	No	Lipostructure	No
B.G.	43	No	Facial atrophy	No	Lipostructure	No
D.I.	53	No	Skin depression	No	Lipostructure	No
D.C.	37	No	Skin depression	No	Lipostructure	No
T.O.	53	Diabetes	Skin scar	No	Lipostructure	No
R.P.	45	No	Skin scar	No	Lipostructure	No
I.P.	31	No	Facial atrophy	No	Lipostructure	No
E.P.	36	No	Skin depression	No	Lipostructure	No
U.E.	27	No	Skin scar	No	Lipostructure	No

Pz, patients.

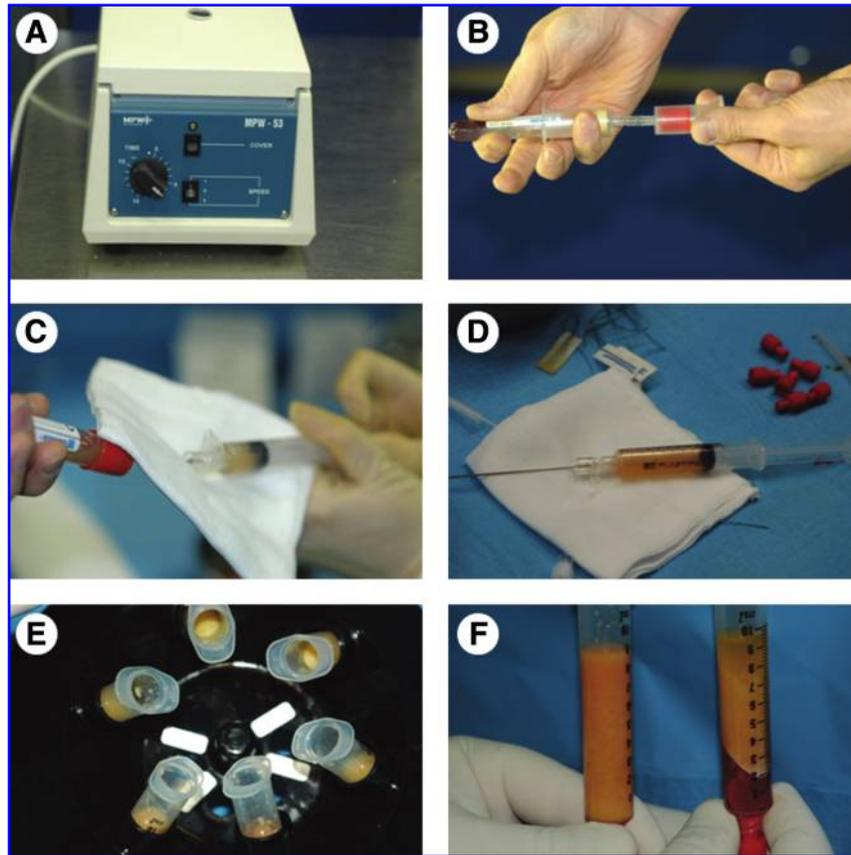


FIG. 1. Method of preparation of platelet-rich plasma (PRP). (A) Cascade-Esforax centrifugation, (B) activation with CaCl_2 , (C) harvest, and (D) final product of 9 cc of activated PRP, (E) sterile centrifuge with syringes after processing for 3 min at 3000 rpm, and (F) syringes with purified fat tissue and fluid fat portion separated for the serous-bloody part.

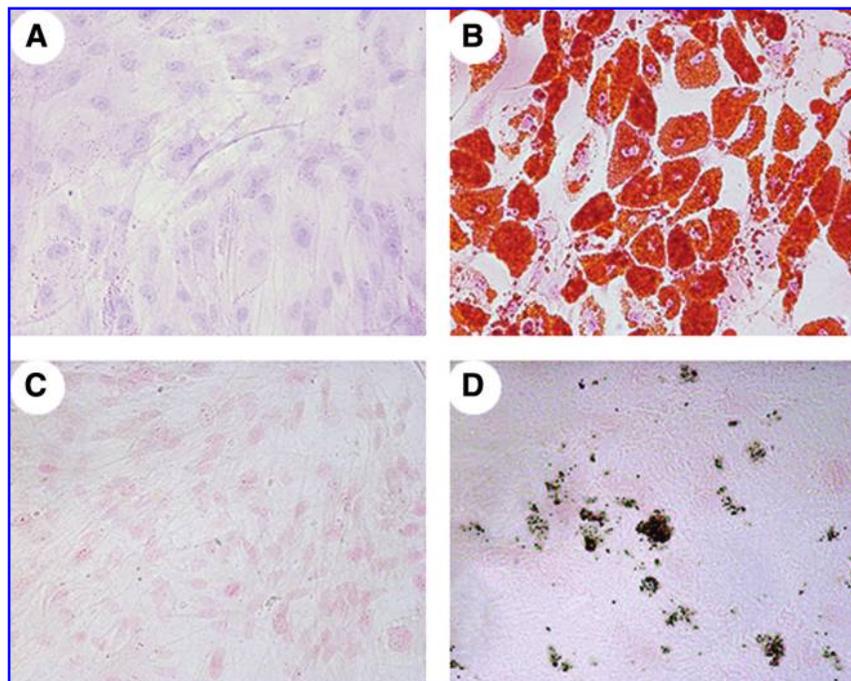


FIG. 2. Adipogenic and osteogenic differentiation of adipose-tissue-derived stem cells (ASCs). Oil Red O staining of (A) control ASCs (DMEM + 10% FBS) and (B) after induction of adipocyte differentiation (see Materials and Methods). Nuclei are counterstained with Hematoxylin. Von Kossa staining in (C) control ASCs (Dulbecco's Modified Eagle's Medium (DMEM) + 10% fetal bovine serum (FBS)) and (D) ASCs after induction of osteogenic differentiation (see Materials and Methods). Nuclei are counterstained with Fast Red.



FIG. 3. Case of a 79-year-old man with foot skin chronic ulcer previously treated with advanced-based collagen and hyaluronic acid for 1 year; ulcer margins were plug with PRP added to centrifuged fat. (A) Preoperative and (B–D) intraoperative views; (E) postoperative situation after 3 weeks and (F) after 4 months.



FIG. 4. Case of a 61-year-old woman, with Romberg syndrome and hemi-facial atrophy, previously treated with plug of polylactic acid and fat grafting without PRP. We plug the subcutaneous region of PRP added to centrifuged fat. (A) Preoperative view in frontal projection and (B) lateral projection. (C) Postoperative frontal projection and (D) lateral projection after 6 months. Postoperative views in (E) frontal and (F) lateral projection after 15 months. Arrows indicate the areas of healing.

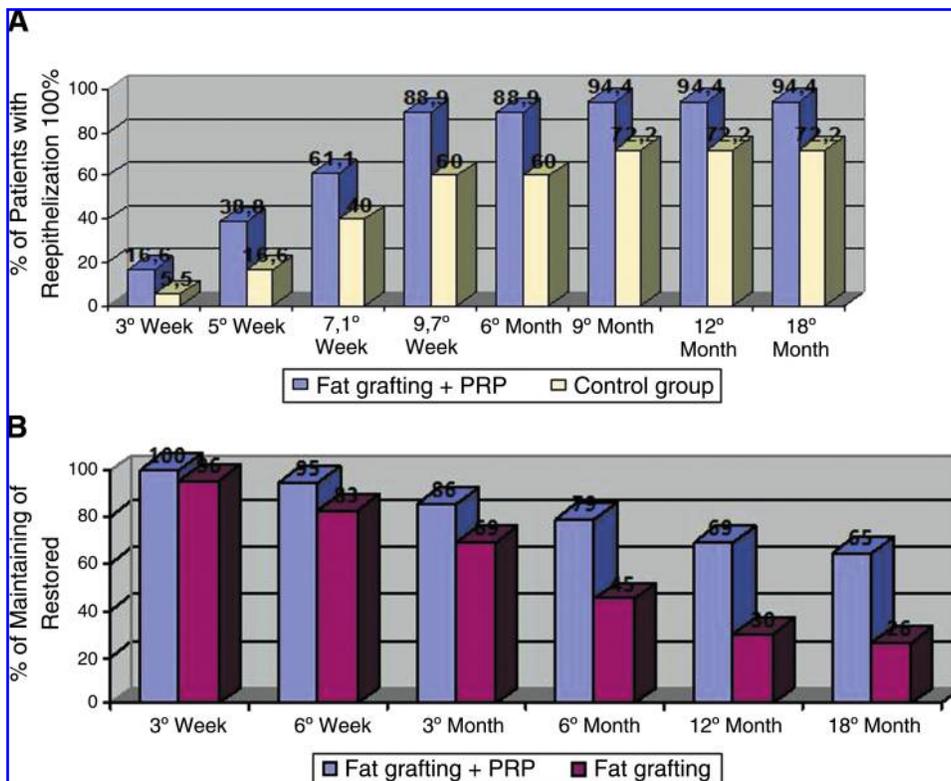


FIG. 5. Bar graphs showing effects of PRP on (A) percentages of skin chronic ulcer reepithelization compared with control group and (B) percentages of maintenance of restored fat compared to control groups. Color images available online at www.liebertonline.com/ten.

eyebrow; (3) reabsorption of fat in one or more regions; (4) time of stabilization of the transplanted fat; (5) need for retreatment.

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

For evaluation of healing rate of skin chronic ulcer, the authors used the method of K. Kazakos,³³ with minor modifications. Briefly, patients were divided into two groups, the first one being made of patients treated with hyaluronic acid and the second one of patients treated by injections of PRP. They then calculate the rate between the initial surface (cm²) and the surface at the end of every week (cm²).

Statistical analysis

Values are shown with standard error of mean as error bars. Results were analyzed by means of Student's *t*-test. The differences were considered statistically significant for $p < 0.05$.

Results

Influence of PRP in tissue regeneration and during fat grafting surgical procedures

The authors observed 61.1% and 88.9% of chronic lower extremity ulcers (Fig. 3A) and 100% reepithelization during an 7.1-week and 9.7-week (average) course of twice-daily

wound treatment with PRP suspended on a collagen base, respectively, compared with 40% and 60% of first control group ($n = 10$), respectively, treated with hyaluronic acid and collagen medication. An example of methods of injection in Figure 3B–D is reported, and clinical results are in Figure 3E and F. We also observed in patients affected by facial disease (Fig. 4A, B) treated with reconstructing three-dimensional projection of face by fat grafting and PRP a 70% maintenance of contour restoring and three-dimensional volume after 1 year, and only 31% in control patients ($n = 10$) treated with only fat grafting. In Figure 4, the clinical efficacy of PRP during fat graft procedures is reported. In Figure 5, the effects of PRP on the maintenance of restored and on reepithelization of skin chronic ulcers compared with respective control groups are summarized.

PRP increases adipose tissue stem cells number in vitro

As reported in Figure 6A, PRP induced an increase of ASC number without any morphological changes compared with control (Fig. 6B, C). There was a statistically significant increase, by around fourfold, at 4 and 6 days, when cells were preconfluent ($p < 0.02$). After 8 days, at confluency, there was a threefold increase of adipose ASC number in PRP cultures compared to controls. Oil Red O staining did not reveal any significant difference in intracytoplasmic lipid accumulation compared with PRP-treated and control ASCs (Fig. 6D, E).

Discussion

The authors demonstrated that the use of PRP mixed with fat resulted in an increase of fat grafting survival and

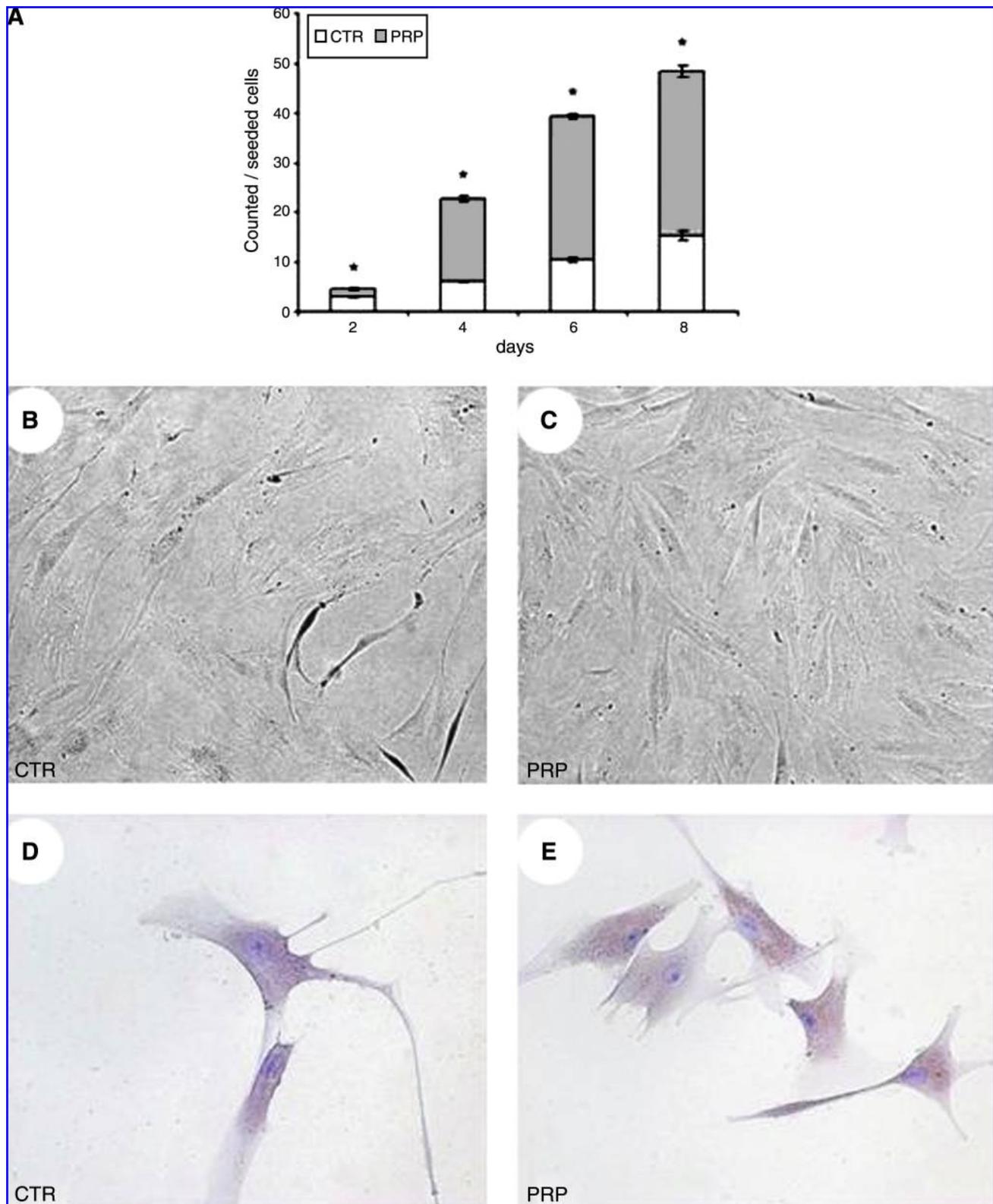


FIG. 6. The effect of PRP on ASC proliferation. **(A)** Bar graph showing growth in control (CTR) and 5% PRP-treated ASCs after 2, 4, 6, and 8 days. **(B, C)** Contrast phase micrographs showing the similar morphological appearance of control (CTR) and PRP-treated cells, at 100 \times magnification. **(D, E)** Oil Red O staining of CTR and PRP-treated ASCs shows a similar intracytoplasmic accumulation of small lipid droplets in both groups, at 200 \times magnification. Color images available online at www.liebertonline.com/ten.

function. We also reported that the use of PRP alone in patients affected by maxillofacial defects produce a regenerative tissue increase. PRP is a mixture of autologous proteins containing 300,000–350,000 platelets/ μL , whose function consists in stimulating dermal fibroblasts when injected.^{2,6}

When 0.5 mL PRP was injected mixed with 1 mL of centrifugated fat, it improved growth and restored fat volume maintenance, confirming data observed with other surgical procedures, including periodontal^{4,5} and oral surgery,^{6–9} maxillofacial⁹ and aesthetic plasticity,^{11–13} spinal fusion,^{14–16} heart bypass surgery,¹⁷ and treatment of soft-tissue ulcers.^{18,19} PRP, being produced during surgical procedures under sterile conditions, is easily produced and safe to use; moreover, PRP lacks surface antigens responsible for potential allergic reaction.³⁴

Our results clearly documented that the use of PRP during fat grafting improves 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.³⁵ In addition, PRP can stimulate neoangiogenic vascularization and fibrogenetic activity of fibroblasts that further improves adipose tissue survival and three-dimensional organization.³⁴

In addition, compared to lipofilling,^{36–38} where fat cells are laid in rows without solution of continuity, implant survival is likely derived from a reduction of fat necrosis due to improved neoangiogenesis in the implanted area. Results of the present *in vivo* tissue-engineering approach suggest two fundamental points. First, PRP sustains an optimal micro-environment that allows correct architectural adipocytes distribution,¹ better cell-to-cell interaction, adipose tissue growth, and differentiation from ASCs; the latter offers early protection from surrounding inflammatory events.³⁹ Second, PRP-induced early development of neoangiogenic micro-capillary network¹¹ facilitates the delivery of proper nutrient and oxygen levels to grafted cells.^{40,41}

In conclusion, our *in vitro* clinical study clearly documented that PRP accelerates skin chronic ulcer reepithelization in patients who underwent plastic and reconstructive surgery and improves maintenance and function of adipose tissue graft in patients who underwent plastic reconstructive surgery, possibly by stimulating ASC proliferation.

Acknowledgment

This work was partially supported by a grant from Transplantation Agency of Lazio, Rome, Italy.

Disclosure Statement

No competing financial interests exist.

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Received: September 17, 2008

Accepted: February 19, 2009

Online Publication Date: April 17, 2009

This article has been cited by:

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