Potentials and Limitations of the Use of Platelet-Rich Plasma (PRP) in Combination with Lipofilling

van Dongen, Joris A.; Stevens, Hieronymus P.; van der Lei, Berend

Published in:
Plastic and Aesthetic Regenerative Surgery and Fat Grafting

DOI:
10.1007/978-3-030-77455-4_23

23

Potentials and Limitations of the Use of Platelet-Rich Plasma (PRP) in Combination with Lipofilling. An Evidence-Based Approach

Joris A. van Dongen, Hieronymus P. Stevens, and Berend van der Lei

Key Messages

- The addition of platelet-rich plasma to lipofilling does not increase skin rejuvenation.
- TCA peeling might be used as a trauma trigger to induce regeneration by platelet-rich plasma enriched lipofilling.
- Platelet-rich plasma enriched lipofilling might be effective to augment dermal wound healing and decrease postoperative recovery time.
- Scientific evidence of platelet-rich plasma as additive to lipofilling to regenerate skin fibrosis is lacking.
- Platelet-rich plasma enriched stromal vascular fraction seems promising as a treatment of alopecia androgenetic although well-designed prospective randomized trials are lacking.
- Well-designed prospective randomized trials are necessary to further develop the field of regenerative medicine in plastic surgery.
- Growth factors released by platelets influence behavior of adipose derived stromal cells.
- Autologous lipofilling can be used as treatment in four different ways: (non)-processed lipofilling, cellular stromal vascular fraction, tissue stromal vascular fraction, or adipose derived stromal cells.

Supplementary Information The online version of this chapter (https://doi.org/10.1007/978-3-030-77455-4_23) contains supplementary material, which is available to authorized users.

J. A. van Dongen (✉)
Department of Plastic and Reconstructive Surgery, University Medical Center Utrecht, Utrecht, the Netherlands

Department of Plastic Surgery, University of Groningen and University Medical Center of Groningen, Groningen, the Netherlands

H. P. Stevens
Velthuis Kliniek, Rotterdam, the Netherlands

B. van der Lei
Department of Plastic Surgery, University of Groningen and University Medical Center of Groningen, Groningen, the Netherlands

Bergman Clinics, Heerenveen, the Netherlands
e-mail: info@berendvanderlei.nl

23.1 Introduction

Lipofilling, the transplantation of adipose tissue, has been used to restore volume loss due to aging and congenital or traumatic defects for decades. Later on, the number of clinical indications for using lipofilling expanded towards a more regenerative based approach, e.g., to remodel dermal fibrosis or to improve dermal wound healing [1]. The regenerative capacity of adipose tissue can be mainly ascribed to the stromal vascular fraction

A. Kalaaji (ed.), *Plastic and Aesthetic Regenerative Surgery and Fat Grafting*, https://doi.org/10.1007/978-3-030-77455-4_23

(SVF). SVF of adipose tissue contains all non-adipocyte cell types, e.g., adipose derived stromal cells (ASCs), fibroblasts, immune cells, endothelial cells as well as extracellular matrix (ECM). One of the most important cell types are ASCs, which reside in the SVF as precursor cells attached to vessels as pericytes and periadventitial cells. In 2001, Zuk et al. were the first to enzymatically isolate SVF from adipose tissue and were able to expand the number of ASCs in culture [2]. ASCs were shown to be capable to differentiate into multiple cell lineages such as ectodermal, endodermal, and mesenchymal cells. Upon culture, ASCs secrete a plethora of growth factors, cytokines as well as proteins, which are immunomodulative and are able to stimulate angiogenesis and remodeling the extracellular matrix. Hence, ASCs are believed to be the key player in the regenerative capacity of SVF from adipose tissue.

To increase the regenerative capacity of lipofilling, ASCs were enzymatically isolated and added to fat used for lipofilling (e.g., the so-called lipograft). The first enzymatic isolation procedure was developed by Zuk et al. and required a specialized culture laboratory (good manufacturing practice facilities), which was expensive and very unpractical for clinical use [2]. In the years following, numerous intra-operative enzymatic isolation devices have been developed. The vast majority of these intra-operative enzymatic isolation devices use collagenase to enzymatically breakdown all adipocytes and cell–cell connections including extracellular matrix resulting in a single cell suspension of SVF cells (cellular SVF (cSVF)). However, these intra-operative enzymatic isolation procedures are still time-consuming and expensive [3]. Moreover, the clinical use of enzymes became forbidden by legislation in many countries. Therefore, other so-called intra-operative non-enzymatic or mechanical isolation procedures were subsequently developed for clinical use. Mechanical isolation procedures only use shear-stress to disrupt adipocytes while maintaining all cell–cell connections including extracellular matrix resulting in a tissue-like SVF (tSVF). These mechanical isolation procedures are less expensive and less time-consuming as compared to enzymatic isolation procedures [3].

Meanwhile, new strategies to improve the regenerative capacity of lipofilling were developed such as platelet-rich plasma (PRP). PRP is defined as a portion of plasma of autologous blood having a concentration of platelets above baseline which can be concentrated in different amounts by several commercially available devices [4]. As an isolated treatment, PRP has been tested for multiple clinical indications such as wound healing or alopecia androgenetic showing some beneficial effect. Platelets synthesize large numbers of growth factors with regenerative capacities, e.g., vascular endothelial growth factor (VEGF), platelet derived growth factor (PDGF), transforming growth factor-β (TGF-β), and epidermal growth factor (EGF). These factors stimulate angiogenesis as well as proliferation and differentiation, e.g., adipogenesis of ASCs and therefore might have a synergistic effect when combined with ASCs present in fat grafts. For instance, VEGF is present in a high amount in fat graft as well as platelets and plays an important role in wound healing by stimulating endothelial proliferation and migration. PDGF stimulates proliferation and migration of ASCs and plays an important role in hair follicular stem cell activity and induction of the anagen phase during the hair cycle in vivo.

Due to the variety of growth factors in platelets influencing ASCs in different ways, the combination of lipofilling with PRP might be even more effective as a treatment for multiple clinical indications, e.g., skin rejuvenation, dermal wound healing, dermal fibrosis, or alopecia androgenetic. Thus, the aim of this chapter is to discuss the current available clinical evidence of the addition of PRP to lipofilling for the aforementioned clinical indications.

23.2 Platelet-Rich Plasma Enriched Lipofilling for Skin Rejuvenation

In 2006, Coleman discovered that lipofilling was more than a permanent filler. Coleman described that the skin above the transplanted adipose tissue improved by showing a decreased pore size and less scarring caused by acne. In the years fol-

lowing, multiple studies described that lipofilling could rejuvenate aged skin [5]. To stimulate skin rejuvenation by lipofilling, PRP was added to lipofilling to function as a biological catalyst for ASCs. Gennai et al. stated that PRP-enriched micro-superficial enhanced fluid fat injection, a form of autologous lipofilling, rejuvenated facial skin in 65 patients (Tables 23.1 and 23.2) [6]. Results were analyzed by subjective clinical assessment of photographs of patients up to till six months post-surgery by the senior author. This study, however, lacks all objective measurement outcomes necessary to analyze effects of skin rejuvenation, statistics and preoperative results. Besides, PRP-enriched lipofilling as single treatment was only used in seven patients. Ozer and Colak treated 14 patients with facial lipofilling mixed with PRP and showed a significant increase in the FACE-Q modules of satisfaction with skin when preoperative scores were compared to postoperative scores with a minimum of 9 months of follow-up [7]. Rigotti et al. was one of the first to show that the addition of PRP to lipofilling histologically changes treated facial skin [8]. This study compared preoperative skin biopsies with 3 months postoperative skin biopsies in 13 patients. PRP-enriched lipofilling resulted in a less organized elastic fiber network with more dissociated elastic fibers with a smaller diameter and smoother surface in the reticular dermis. Furthermore, PRP-enriched lipofilling also improved vasculature and increased inflammation in the postoperative skin biopsies. Although this study showed interesting histological changes of the skin after treatment of PRP-enriched lipofilling, no clinical results were mentioned nor described (Tables 23.1 and 23.2) [8]. Therefore, it remains unclear whether these histological changes are significant enough to lead to visible skin changes and/or improvement. Moreover, all the aforementioned studies did not use a control group. The lack of a control group implicates getting insufficient reliable results because a placebo effect cannot be excluded. It is also well-known that fine needling can induce skin changes, another reason definitely to include a control group.

To date, only one well-designed prospective randomized double-blinded placebo-controlled clinical trial has been published studying the addition of PRP to facial lipofilling to rejuvenate aged skin (Tables 23.1 and 23.2) [9]. This study treated 25 patients divided into two treatment groups: saline enriched lipofilling versus PRP-enriched lipofilling. Patients were followed for 12 months and results were analyzed using a validated cutometer to measure skin elasticity. Skin elasticity in both groups did not improve when preoperative skin was compared with postoperative skin nor did the addition of PRP result in an improved skin elasticity postoperative [9]. However, when a regression analysis was performed of skin elasticity as a function of age, a negative correlation was seen preoperative in both intervention groups. After treatment of lipofilling or PRP-enriched lipofilling, the correlation reverses, especially in the PRP-enriched lipofilling group. Although the results were not significant, this could be an indication of some skin rejuvenation. Furthermore, the recovery time expressed as the number of days it took to return to work was significantly lower when patients underwent PRP-enriched facial lipofilling in comparison with saline enriched facial lipofilling [9]. These results indicate that the addition of PRP accelerates the recovery of damaged tissue that is caused during the intervention. The addition of PRP therefore might be more beneficial in case of restoring damaged tissue, e.g., fibrosis or wound healing, instead of rejuvenation of relatively healthy skin. To date, the evidence of PRP-enriched lipofilling as a treatment for skin rejuvenation is thus lacking although postoperative recovery time is significantly reduced.

23.3 Platelet-Rich Plasma Enriched Lipofilling for Wound Healing

Chronic non-healing dermal wounds are a large socioeconomical burden worldwide. In 2018, the prevalence of chronic wounds of mixed etiologies, i.e., venous ulcers, diabetic ulcers, arterial

Table 23.1 Clinical data of studies using PRP-enriched lipofilling as a treatment for skin rejuvenation purposes

Reference	Study type	Study population	Intervention	Follow-up	Results	Complications
Gennai et al. (2017)	Retrospective, non-controlled, non-blinded, non-randomized	Patients with aging skin in periocular and perioral region ($n = 65$).	Intervention: M-SEFFI + PRP Co = intervention: SEFFI or MIVEL or blepharoplasty	Clinical photographs evaluated by senior author and patients using a non-validated method up to 6 months postoperative	No preoperative results. No statistics used	No complications reported
Ozer and Colak (2019)	Prospective, non-controlled, non-blinded, non-randomized	Patients with aging facial skin ($n = 14$).	Intervention: MAFT + PRP	FACE-Q modules at least 9 months postoperative	***Satisfaction with skin improved from 33.7 ± 18.1 to 88.0 ± 20.3	No complications reported
Rigotti et al. (2016)	Prospective, controlled, non-blinded, non-randomized	Patients with aging skin in preauricular region ($n = 13$).	Intervention: Lipofilling + PRP	Histological analysis 3 months postoperative	Reticular dermis showed a decrease in elastic fibers with reduced diameter and smoother. Increase of mononuclear cell infiltration around vessels. No statistics used	Not mentioned
Willemsen et al. (2018)	Prospective, controlled, blinded, randomized	Patients with aging facial skin ($n = 25$)	Intervention: Lipofilling + PRP Control: Lipofilling + NaCl	Skin elasticity measured with the cutometer up to 12 months postoperative	No significant difference in skin elasticity between both groups and within each group when preoperative is compared to postoperative	No complications reported

PRP platelet-rich plasma, *M-SEFFI* micro-superficial enhanced fluid fat injection, *SEFFI* superficial enhanced fluid fat injection, *MIVEL* minimal incision vertical endoscopic lift, *MAFT* micro-autologous fat transplantation

$^{***}p < 0.001$

Table 23.2 Methodology data of processing fat and PRP in clinical studies using PRP-enriched lipofilling as a treatment for skin rejuvenation purposes

Reference	Fat harvesting	Fat processing	Volume of fat	PRP processing	Coagulant	Volume of PRP	Activation of platelets	Increase of platelets
Gennai et al. (2017)	15 cm long multi-perforated cannula with 0.3 mm-diameter ports	Decantation (two times)	Fat/PRP: Mean 7.3 mL (range: 0.6–29 m)	18 mL of whole blood centrifuged at 2000 rpm for 4 min	Citrate	Fat/PRP: Mean 7.3 mL (range: 0.6–29 m)	Not mentioned	Not measured
Ozer and Colak (2019)	20 cm long multiport cannula with blunt tip with 24, 1 mm-diameter side holes	Decantation, centrifugation at 300 ×*g* for 4 min	35.6 mL	132 mL of whole blood centrifuged at 1650 ×*g* for 5 min	Citrate	14.4 mL	No	4-fold
Rigotti et al. (2016)	15 cm long cannula with 3 distal holes	Decantation	1 mL	Whole blood centrifuged at 300 ×*g* for 5 min. Then, centrifuged at 700 ×*g* for 17 min. After activation, centrifuged at 3000 ×*g* for 20 min	Citrate	1 mL	$CaCl_2$ and thrombin	5.4–7.3 fold
Willemsen et al. (2018)	22 cm long cannula with a 2.4 cm diameter	Standard Coleman	36 mL	30 mL of whole blood processed according to the Biomet GPSIII	Citrate	3 mL	No	3–6 fold

PRP platelet-rich plasma, *$CaCl_2$* calcium chloride

ulcers, and pressure ulcers was 2.21 per 1000 population [10]. In the USA only, chronic wounds affect over six million cases with an annual cost of more than 20 billion every year [11]. To date, many conventional wound healing treatments are costly and ineffective for the vast majority of patients. Hence, the need for new effective therapeutic modalities is increasing. In chronic non-healing dermal wounds, parenchymal skin tissue is damaged and needs to be regenerated. As mentioned in the introduction, ASCs in fat graft have shown to be able to (in)directly contribute to parenchymal regeneration by differentiation and secretion of large numbers of trophic factors that stimulate processes, e.g., angiogenesis, immunomodulation, and remodeling of extracellular matrix which are vital for proper dermal wound healing. Thus far, several clinical trials using ASCs or non-dissociated adipose tissue, i.e., fat grafting, have shown to be a promising new therapy to augment dermal wound healing. To further enhance the regenerative effect of lipofilling on dermal wound healing, PRP was added. Till now, only a few clinical studies investigated the combined effect of lipofilling with PRP on dermal wound healing.

One prospective randomized clinical trial treated 24 patients with chronic dermal ulcers were treated by standard wound care: disinfection, debridement when needed, and application of advanced wound dressing as well as bandage (Tables 23.3 and 23.4) [12]. Sixteen patients were treated by standard wound care and one injection of mechanically isolated ASCs with PRP while the control group only received standard wound care. Although, no formal proof was given of the presence of ASCs in the mechanically isolated fraction. In both groups, similar number of wounds healed completely, respectively, 68% in the control group and 71% in the experimental group. Nevertheless, the wound closure rate was significantly faster with 0.2287 cm^2/day in the experimental group in comparison with 0.0890 cm^2/day in control group ($p = 0.0257$). No major complications were mentioned in both groups. Previous studies have shown that lipofilling without additive might improve and/or accelerate wound healing in chronic dermal ulcers. In this study, no control group using lipofilling or PRP alone to treat chronic dermal ulcers was used and thus it remains unknown whether the addition of PRP to lipofilling improves the regenerative effect of lipofilling.

Another research group performed three case series treating lower extremity ulcers with PRP combined with lipofilling (Tables 23.3 and 23.4). In the first case series, 20 patients with lower extremity ulcers were treated with a PRP/lipofilling mixture and ten patients received a hyaluronic acid injection [13]. Sixteen out of 20 patients had complete ulcer healing after an average of 9.7 weeks, while five out of ten patients had complete ulcer healing after 8.4 weeks on average. The second study by the same research group treated 30 patients with lower extremity ulcers with PRP mixed with lipofilling and showed complete healing in 57% of the patients after 3 months [14]. Both studies lack a proper control group with only a treatment of lipofilling or PRP as well. Hence, the effect of the addition of PRP to lipofilling on lower extremity ulcers remains unclear. The third study compared PRP-enriched lipofilling, PRP only, cSVF enriched lipofilling and hyaluronic acid to treat 40 patients with post-traumatic lower extremity ulcers [15]. PRP as well as cSVF enriched lipofilling showed similar re-epithelization rates, respectively, 97.8% ± 1.5% and 97.9% ± 1.5% after 9.7 weeks. PRP only group showed less re-epithelization after 9.7 weeks with 89.1% ± 3.8%, while the hyaluronic acid group showed a re-epithelization rate of 87.8% ± 4.4%. This indicates that PRP-enriched lipofilling is more effective in augmentation of dermal wound healing as compared to PRP only. However, it still remains unclear whether PRP enhances the regenerative capacity of lipofilling regarding wound healing. Well-designed prospective randomized clinical trials treating chronic dermal wounds with PRP-enriched lipofilling can only elucidate this aspect.

Table 23.3 Clinical data of studies using PRP-enriched lipofilling as a treatment for wound healing

Reference	Study type	Study population	Intervention	Follow up	Results	Complications
Raposio et al. (2017)	Prospective, controlled, non-blinded, randomized	Patients with chronic skin ulcers ($n = 40$). Control: Mean age = 14.5 months. Intervention: Mean age = 26.6 months	Intervention: Standard wound care + ASCs + PRP Control: Standard wound care	Wound closure rate evaluated up to 18 months postoperative	No difference in number of wounds healed. *Faster wound closure rate in the intervention group respectively 0.2287 cm^2/day vs 0.089 cm^2/day	No complications reported
Cervelli et al. (2009)	Prospective, controlled, non-blinded, non-randomized	Patients with lower extremity chronic ulcers and vascular disease ($n = 20$)	Intervention: Lipofilling + PRP Control: Medication based collagen and hyaluronic acid	Wound closure rate evaluated up to 12 months postoperative	Intervention: 80% of the ulcers healed after 9.7 weeks on average. Control: 50% of the ulcers healed after 8.4 weeks on average	Not mentioned
Cervelli et al. (2010)	Prospective, non-controlled, non-blinded, non-randomized	Patients with ulcers or substances loss of the lower limb ($n = 30$)	Intervention: Lipofilling + PRP + hyaluronic acid	Wound closure rate evaluated up to 3 months postoperative. Biopsies taken 15 days postoperative	Complete healing occurred in 57% of the patients. Increase in cell proliferation was noted without mentioning quantitative data	Two infections
Cervelli et al. (2011)	Prospective, controlled, non-blinded, non-randomized	Patients with posttraumatic lower extremity ulcers ($n = 40$)	Intervention 1: PRP + lipofilling Intervention 2: cSVF + lipofilling Control 1: Hyaluronic acid Control 2: PRP gels	Wound closure rate evaluated up to 10 weeks postoperative	Intervention 1: *Closure rate 97.8 ± 1.5%, Intervention 2: *Closure rate 97.9 ± 1.5%, Control 1: *Closure rate 87.8 ± 4.4%, Control 2: *Closure rate 89.1 ± 3.8%	Three infections

ASCs adipose derived stromal cells, *PRP* platelet-rich plasma, *cSVF* cellular stromal vascular fraction
*$p < 0.05$

Table 23.4 Methodology data of processing fat and PRP in clinical studies using PRP-enriched lipofilling as a treatment for wound healing

Reference	Fat harvesting	Fat processing	ASCs/SVF isolation	Number of cells	Volume of fat	PRP processing	Coagulant	Volume of PRP	Activation of platelets	Increase of platelets
Raposio et al. (2017)	3.0 blunt-tipped cannula	Not applicable	Mechanical	$5 * 10^5$	Not applicable	42 ml of whole blood centrifuged at 210 *xg* for 10 min.	Citrate	5 ml	Thrombin	4–seven fold
Cervelli et al. (2009)	1.5, 2 and 3 mm diameter cannulas	Centrifugation at 3000 rpm for 3 min	Not applicable	Not applicable	0–25 ml	18 ml of whole blood centrifuged at 1100 *xg* for 10 min. using the Cascade kit.	Not mentioned	4–50 ml	$CaCl_2$	Not mentioned
Cervelli et al. (2010)	Coleman	Centrifugation at 3000 rpm for 3 min	Not applicable	Not applicable	Not mentioned	16 ml of whole blood centrifuged at 1500 *xg* using the Regen kit.	Not mentioned	Not mentioned	Thrombin	Not mentioned
Cervelli et al. (2011)	3 mm cannulas	Centrifugation at 3000 rpm	Enzymatic	Not mentioned	1/0.3–05 ratio fat/ PRP	18 ml of whole blood and centrifuged at 1100 *xg* for 15 min. using the Cascade kit.	Not mentioned	1/0.3–05 ratio fat/ PRP	$CaCl_2$	Not mentioned

ASCs adipose derived stromal cells, *SVF* stromal vascular fraction, *PRP* platelet-rich plasma, *$CaCl_2$* calcium chloride

23.4 Platelet-Rich Plasma Enriched Lipofilling for Dermal Fibrosis

Fibrosis of the skin occurs after burning and other types of wound healing or can be caused by systemic or local diseases of the skin, e.g., scleroderma or lichen sclerosis. Skin fibrosis can be cosmetically disfiguring because of hyper- or hypopigmentation as well as increased stiffness, roughness, or irregularity. Mostly skin fibrosis has both a significant social and behavioral impact. Furthermore, severe skin fibrosis can be associated with pain and decreased functionality, depending on the anatomical location. For instance, fibrosis in the perioral region will affect the opening of the mouth, as is, e.g., often the case in scleroderma patients [16]. Fibrosis in the genital region, i.e., lichen sclerosis, causes pain, sexual and urinal dysfunction, and an increased risk of vulvar squamous cell carcinoma. This is caused by a chronic state of inflammation, decreased angiogenesis resulting in epithelial damage and extracellular matrix accumulation but the exact mechanism remains unclear [17, 18]. To date, no gold standard treatment exists for systemic or local fibrotic disease of the skin or scarring due to wound healing or burn wounds.

Lipofilling has become a new treatment modality for scar tissue; it seems to have beneficial effects on scar tissue by decreasing pain and improving scar appearance. Yet, well-designed prospective randomized clinical trials have to be performed, especially for treatment of scleroderma and lichen sclerosis. The combination of PRP and lipofilling has only been investigated in a few clinical studies and case reports for treatment of scleroderma, lichen sclerosis, posttraumatic and burn wound scars (Tables 23.5 and 23.6). One study used PRP-enriched cSVF to treat six patients with scleroderma in the facial area [16]. Perioral and malar areas showed an increase in skin elasticity of, respectively, 16.64% and 17.80% measured with a skin elastometer, with a follow-up till 3 months. In five out of six patients, the opening of the mouth showed a significant increase. Videodermatoscope examination of the perioral vasculature displayed a significant growth of capillary density in four out of six patients. Complications were not mentioned [16]. A case report by Kim et al. reported a treatment with PRP-enriched lipofilling in a female patient of 67-year-old with severe symptoms of lichen sclerosis for more than 5 years [19]. Forty ml of PRP-enriched lipofilling was injected in the subcutaneous layer of the labia majora. One year after treatment, the patient revealed no signs of vaginal pruritus or irritation anymore and the contour of the labia majora was restored. Classical characteristic white patchy lesions of lichen sclerosis were significantly diminished and did not recur in the 1 year of follow-up.

A larger prospective three-armed clinical study was performed treating ten patients in each arm with PRP-enriched lipofilling (Tables 23.5 and 23.6) [20]. SVF-enriched lipofilling and lipofilling for posttraumatic scars and burn wound scars. The study had no randomization nor was blinded. Volume preservation was 69%, 63%, and 39% for, respectively, PRP-enriched lipofilling, SVF-enriched lipofilling, and lipofilling group after 1 year. However, these volume preservation measurements were subjectively assessed by the authors based on just clinical photographs. All patients were reported to be satisfied with their scar appearance after treatment. No data were presented with regard to the pre- and postoperative scar appearance. Therefore, no reliable conclusions can be drawn from this prospective study because of lack of objective validated measurements [20].

Although lipofilling seems to be beneficial as a treatment modality for skin fibrosis, thus far scientific evidence of the beneficial effect of adding PRP to the lipograft definitely is lacking.

23.5 Platelet-Rich Plasma Enriched Lipofilling for Alopecia Androgenetic

Alopecia androgenetic is characterized by progressive hair loss, which is genetically determined. Its prevalence increases with age. Pathogenesis of alopecia androgenetic is illus-

Table 23.5 Clinical data of studies using PRP-enriched lipofilling as a treatment for dermal fibrosis

Reference	Study type	Study population	Intervention	Follow up	Results	Complications
Virzi et al. (2017)	Prospective, non-controlled, non-blinded, non-randomized	Patients with systemic sclerosis ($n = 6$)	Intervention cSVF + PRP	Skin elasticity (Elastometer-EM 25) and extension indices of the labial rhyme evaluated up to 3 months postoperative	Substantial increase in average skin elasticity of 16.64% in lip area and 17.80% in cheek area as well as improvement of labial rhyme extension	Not mentioned
Kim et al. (2017)	Case report	Patients with lichen sclerosis	Intervention: Lipofilling + PRP	Patients' perception evaluated up to 1 year postoperative	No signs of vaginal pruritus or irritation. White patchy lesion disappeared	No complications
Gentile et al. (2014)	Prospective, controlled, non-blinded, non-randomized	Patients with facial scars ($n = 20$)	Intervention 1: Lipofilling + PRP Intervention 2: Lipofilling + cSVF Control: Lipofilling	Subjective observation up to 60 months postoperative	No data were presented with regard to the pre- and postoperative scar appearance	No complications

cSVF cellular stromal vascular fraction, *PRP* platelet-rich plasma

Table 23.6 Methodology data of processing fat and PRP in clinical studies using PRP-enriched lipofilling as a treatment for dermal fibrosis

Reference	Fat harvesting	Fat processing	ASCs/SVF isolation	Number of cells	Volume of fat	PRP processing	Coagulant	Volume of PRP	Activation of platelets	Increase of platelets
Virzi et al. (2017)	10 gauge cannula	Centrifugation at 2700 rpm for 5 min	Enzymatic	Not mentioned	92–120 mL	20–25 mL of whole blood processed according to the ACP Arthrex system	Citrate	9–12 mL	Not mentioned	Not mentioned
Kim et al. (2017)	Two-hole cannula with blunt tip	Not mentioned	Not applicable	Not applicable	36 mL	30 mL of whole blood processed according to the SmartPrep® APC-30	Not mentioned	4 mL	Not mentioned	Not mentioned
Gentile et al. (2014)	Coleman technique	Centrifugation at 3000 rpm for 3 min	Enzymatic	Not mentioned	1 mL	18 mL of whole blood processed according to the Cascade-Selphyl-aesthetic factors system	Sodium citrate	0.5 mL	Ca^{2+}	Not mentioned

ASCs adipose derived stromal cells, *SVF* stromal vascular fraction, *PRP* platelet-rich plasma, *APC* autologous platelet concentrate, Ca^{2+} calcium

trated by gradual transformation of terminal hairs (i.e., a thick, long, and dark hair) into vellus hairs (i.e., a short, thin, and slightly colored hair) [21]. This process is caused by modifications in the hair cycle, such as shortening of the telogenic and anagenic stages, resulting in a decreased number of both terminal and vellus hairs [22]. Modifications in the hair cycle are also influenced by circulating androgen hormones [23]. Currently available conventional and surgical treatments are limited effective with high costs and side effects, such as long-life use of medication. Till now, several studies have been performed investigating the use of PPR or lipofilling to treat alopecia androgenetic. Although these studies have demonstrated promising result, scientific evidence in form of well-designed prospective randomized clinical trials is still lacking. The use of PRP-enriched lipofilling for alopecia androgenetic has been tested in two case series. The first case series published by Stevens et al. used PRP-enriched tSVF to treat ten male patients characterized by stage II and III alopecia androgenetic according to the Norwood–Hamilton scale (Tables 23.7 and 23.8) [24]. After 12 weeks, mean hair density was significantly increased as measured by a trichoscan epiluminescence microscopy. However, no control group was used and no report was made concerning complications or adverse events.

Another study published by Butt et al. treated 22 patients suffering from alopecia androgenetic with either PRP (n = 11) or PRP-enriched cSVF (Tables 23.7 and 23.8) [25]. All male patients were characterized by alopecia androgenetic class III to VI according to the Norwood–Hamilton scale while all female patients were characterized by alopecia androgenetic class I to III according to the Ludwig score. Hair density was also measured by trichoscan epiluminescence microscopy. In contrast to the previous study, all patients were treated twice with an interval of 4 weeks. Mean hair density increased from 37.66 ± 7.43 to 57.11 ± 7.73 hair/cm^2 and from 52.44 ± 9.66 to 63.72 ± 11.68 hair/cm^2 after 6 months, respectively, in the PRP-enriched cSVF and PRP group. The authors concluded that PRP-enriched cSVF treatment is more effective than solely PRP in reversing the effects of alopecia androgenetic. However, mean preoperative hair density scores showed a large difference between the PRP-enriched cSVF and PRP group with a lower hair density score for the PRP-enriched cSVF group [25]. This observation

Table 23.7 Clinical data of studies using PRP-enriched lipofilling as a treatment for alopecia androgenetic

Reference	Study type	Study population	Intervention	Follow-up	Results	Complications
Stevens et al. (2018)	Prospective, non-controlled, non-blinded, non-randomized	Patients with alopecia androgenetic (n = 6)	tSVF + PRP	Hair density was evaluated using the fotofinder epiluminescence microscopy up to 12 weeks postoperative	***Hair density increased with an average of 30.7 hairs/cm^2	Not mentioned
Butt et al. (2019)	Prospective, controlled, non-blinded, non-randomized	Patients with alopecia androgenetic (n = 22)	Intervention: cSVF + PRP Control: PRP	Hair density was evaluated using the fotofinder epiluminescence microscopy up to 6 months postoperative	***Hair density was higher in intervention group (51.64) versus control group (21.51%)	Not mentioned

tSVF tissue stromal vascular fraction, *PRP* platelet-rich plasma, *cSVF* cellular stromal vascular fraction
$^{***}p < 0.001$

Table 23.8 Methodology data of processing fat and PRP in clinical studies using PRP-enriched lipofilling as a treatment for alopecia androgenetic

Reference	Fat harvesting	Fat processing	ASCs/SVF isolation	Number of cells	Volume of SVF	PRP processing	Coagulant	Volume of PRP	Activation of platelets	Increase of platelets
Stevens et al. (2018)	Not mentioned	Not applicable	Mechanical	1.275×10^6	1 mL	15 mLof whole blood processed according to the ACP Arthrex system	Not used	5 mL	Not mentioned	Not mentioned
Butt et al. (2019)	3 mm cannula	Not applicable	Enzymatic	100,000	Not applicable	9 mL of whole blood processed according to the tray life tube gel kit	Citrate	3 mL	Not mentioned	Not mentioned

ASCs adipose derived stromal cells, *SVF* stromal vascular fraction, *PRP* platelet-rich plasma

implicates that both study groups were not comparable to each other; a lower hair density score in the PRP-enriched cSVF group indicates a higher potential of regeneration.

Despite promising results of the just mentioned two studies, no hard conclusions can be made upon the potential effect of PRP-enriched cSVF/tSVF as a treatment for alopecia androgenetic. It remains unclear whether the addition of PRP to cSVF/tSVF or vice versa is necessary to obtain maximum regeneration of hairs because the first study only used one study arm and the second study failed to treat a comparable group of patients.

23.6 Practical Experience of Using Platelet-Rich Plasma

PRP was introduced in cosmetic surgery practice of the senior author back in January 2009. Initially, PRP was tested for its practical use in lipofilling of the buttock. In 2013 results of a consecutive case series were published showing that volumes for lipofilling could be doubled without an increase of complications like oil cyst formation. It was felt save now to also use PRP in the face together with lipofilling to investigate whether the theoretical relevance of improved graft survival could be reconfirmed. Up to date over 1000 cases of the combination of PRP with lipofilling have been performed. Since 2015, when fractionation of lipoaspirate was finally turning into a reproducible procedure for the making of tSVF, PRP was added. The hypothetical regeneration potential of the combination of PRP and tSVF has led to the treatment of various conditions such as arthritic joints (*manuscript submitted*), perianal fistulas (*manuscript submitted*), and aging of facial skin (*in preparation*). Positive results of the studies regarding arthritic joints and perianal fistulas were shown. However, it should be mentioned that all aforementioned studies are case series and formal proof by results of randomized clinical trials should follow. As an anti-aging treatment, this technique added to lipofilling seems to yield less impressive results, possibly because the required "trauma trigger" is not present. This hypothesis seems even more likely as, from our own personal experience, in combination with TCA-peeling results of superficial lipofilling plus PRP do seem to give improvement on the level of the skin by reducing dark spots, reducing pore size reducing the number and depth of fine wrinkles. The concept of "chemical splinting" is hereby introduced, the combination of immobilization of the skin (potentially allowing for better repair of superficial cellular damage of the skin) plus adding sufficient trauma to the area where tSVF potentially has to be triggered above a minimum level before getting "activated" and starting to "perform." Altogether, PRP has become an important element in regenerative plastic surgery, as long as it is used for the appropriate indication (Fig. 23.1).

23.7 Conclusion

Thus far, no hard clinical evidence exists whether the addition of PRP to lipofilling or the addition of any regenerative component of adipose tissue, e.g., ASCs, cSVF and tSVF is beneficial for skin rejuvenation. Several case series in literature as well as our own clinical experience suggest a beneficial effect of the addition of PRP to lipofilling and tSVF for wound healing purposes and alopecia androgenetic although formal proof has yet to be given. In the future, well-designed prospective clinical trials investigating the real effect of lipofilling in case of skin fibrosis, wound healing, and alopecia androgenetic should therefore first be undertaken before widespread clinical application.

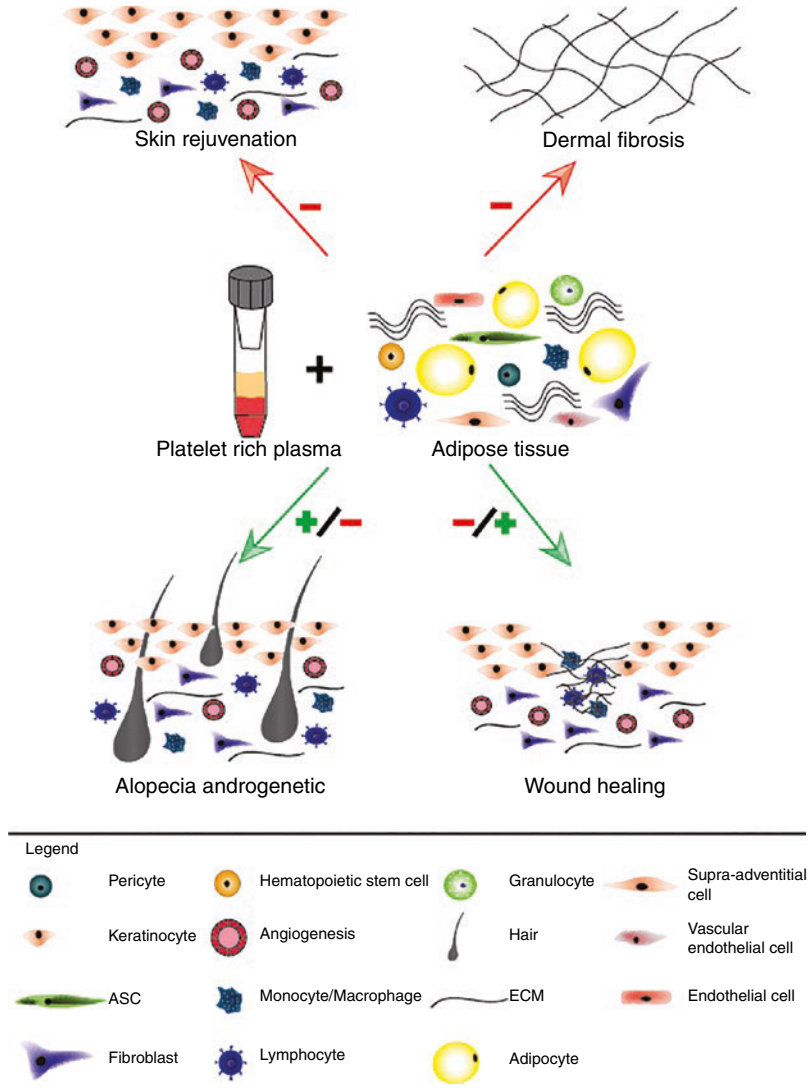

Fig. 23.1 Schematic overview of indications and contra-indications of platelet-rich plasma enriched lipofilling or any subcomponent of adipose tissue, i.e., tissue stromal vascular fraction, cellular stromal vascular fraction, or adipose derived stromal cells. − contraindication of platelet-rich plasma enriched lipofilling, +/− platelet-rich plasma enriched lipofilling might be effective although well-designed prospective randomized placebo-controlled clinical trials are lacking, *ASC* adipose derived stromal cell, *ECM* extracellular matrix

References

1. Negenborn VL, Groen JW, Smit JM, Niessen FB, Mullender MG. The use of autologous fat grafting for treatment of scar tissue and scar-related conditions: a systematic review. Plastic Reconstruct Surg. 2016;137(1):31e–43e.
2. Zuk PA, Zhu M, Mizuno H, et al. Multilineage cells from human adipose tissue: implications for cell-based therapies. Tissue Eng. 2001;7(2):211–28.
3. van Dongen JA, Tuin AJ, Spiekman M, Jansma J, van der Lei B, Harmsen MC. Comparison of intraoperative procedures for isolation of clinical grade stromal vascular fraction for regenerative purposes: a systematic review. J Tissue Eng Regen Med. 2017;
4. Fibel KH, Hillstrom HJ, Halpern BC. State-of-the-art management of knee osteoarthritis. World J Clin Cases. 2015;3(2):89–101.
5. van Dongen JA, Langeveld M, van de Lande LS, Harmsen MC, Stevens HP, van der Lei B. The effects of facial lipografting on skin quality: a systematic review. Plastic Reconstruct Surg. 2019;144(5):784e–97e.
6. Gennai A, Zambelli A, Repaci E, et al. Skin rejuvenation and volume enhancement with the micro superficial enhanced fluid fat injection (M-SEFFI) for skin aging of the periocular and perioral regions. Aesthet Surg J. 2017;37(1):14–23.
7. Ozer K, Colak O. Micro-autologous fat transplantation combined with platelet-rich plasma for facial filling and regeneration: a clinical perspective in the shadow of evidence-based medicine. J Craniofac Surg. 2019;30(3):672–7.

8. Rigotti G, Charles-de-Sa L, Gontijo-de-Amorim NF, et al. Expanded stem cells, stromal-vascular fraction, and platelet-rich plasma enriched fat: comparing results of different facial rejuvenation approaches in a clinical trial. Aesthet Surg J. 2016;36(3):261–70.
9. Willemsen JCN, Van Dongen J, Spiekman M, et al. The addition of platelet-rich plasma to facial Lipofilling: a double-blind, placebo-controlled, randomized trial. Plast Reconstr Surg. 2018;141(2):331–43.
10. Martinengo L, Olsson M, Bajpai R, et al. Prevalence of chronic wounds in the general population: systematic review and meta-analysis of observational studies. Ann Epidemiol. 2019;29:8–15.
11. Hanson SE, Bentz ML, Hematti P. Mesenchymal stem cell therapy for nonhealing cutaneous wounds. Plast Reconstr Surg. 2010;125(2):510–6.
12. Raposio E, Bertozzi N, Bonomini S, et al. Adipose-derived stem cells added to platelet-rich plasma for chronic skin ulcer therapy. Wounds. 2016;28(4):126–31.
13. Cervelli V, Gentile P, Grimaldi M. Regenerative surgery: use of fat grafting combined with platelet-rich plasma for chronic lower-extremity ulcers. Aesthet Plast Surg. 2009;33(3):340–5.
14. Cervelli V, De Angelis B, Lucarini L, et al. Tissue regeneration in loss of substance on the lower limbs through use of platelet-rich plasma, stem cells from adipose tissue, and hyaluronic acid. Adv Skin Wound Care. 2010;23(6):262–72.
15. Cervelli V, Gentile P, De Angelis B, et al. Application of enhanced stromal vascular fraction and fat grafting mixed with PRP in post-traumatic lower extremity ulcers. Stem Cell Res. 2011;6(2):103–11.
16. Virzi F, Bianca P, Giammona A, et al. Combined platelet-rich plasma and lipofilling treatment provides great improvement in facial skin-induced lesion regeneration for scleroderma patients. Stem Cell Res Ther. 2017;8(1):236.
17. Kaya G, Augsburger E, Stamenkovic I, Saurat JH. Decrease in epidermal CD44 expression as a potential mechanism for abnormal hyaluronate accumulation in superficial dermis in lichen sclerosus et atrophicus. J Invest Dermatol. 2000;115(6):1054–8.
18. Gambichler T, Skrygan M, Czempiel V, et al. Differential expression of connective tissue growth factor and extracellular matrix proteins in lichen sclerosus. J Eur Acad Dermatol Venereol. 2012;26(2):207–12.
19. Kim SH, Park ES, Kim TH. Rejuvenation using platelet-rich plasma and lipofilling for vaginal atrophy and Lichen Sclerosus. J Menopausal Med. 2017;23(1):63–8.
20. Gentile P, De Angelis B, Pasin M, et al. Adipose-derived stromal vascular fraction cells and platelet-rich plasma: basic and clinical evaluation for cell-based therapies in patients with scars on the face. J Craniofac Surg. 2014;25(1):267–72.
21. Whiting DA. Diagnostic and predictive value of horizontal sections of scalp biopsy specimens in male pattern androgenetic alopecia. J Am Acad Dermatol. 1993;28(5 Pt 1):755–63.
22. Stough D, Stenn K, Haber R, et al. Psychological effect, pathophysiology, and management of androgenetic alopecia in men. Mayo Clin Proc. 2005;80(10):1316–22.
23. Kaliyadan F, Nambiar A, Vijayaraghavan S. Androgenetic alopecia: an update. Indian J Dermatol Venereol Leprol. 2013;79(5):613–25.
24. Stevens HP, Donners S, de Bruijn J. Introducing platelet-rich stroma: platelet-rich plasma (PRP) and stromal vascular fraction (SVF) combined for the treatment of androgenetic alopecia. Aesthet Surg J. 2018;38(8):811–22.
25. Butt G, Hussain I, Ahmad FJ, Choudhery MS. Stromal vascular fraction-enriched platelet-rich plasma therapy reverses the effects of androgenetic alopecia. J Cosmet Dermatol. 2020;19(5):1078–85.