Chapter 4 - The addition of PRP to facial lipofilling: a double-blind placebo-controlled randomized trial

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Chapter 4 - The addition of PRP to facial lipofilling: a double-blind placebo-controlled randomized trial

Introduction

Lipofilling is a treatment modality to restore tissue volume, but may also rejuvenate the aging skin. Platelet-rich plasma has been reported to augment the efficacy of lipofilling, both on graft take and rejuvenation by altering the ADSC. Authors hypothesized that PRP addition would increase the rejuvenating effect while shortening recovery time.

Material and Methods

The study conducted was a single-centre, double blinded, placebo-controlled randomized trial (2012-2015). In total, a well-defined cohort of 32 healthy females enrolled in the study, with 25 completing the follow-up. All patients underwent aesthetic facial lipofilling with either saline or PRP added. Outcome was determined by changes in skin elasticity, volumetric changes of the nasolabial fold, recovery time and patient satisfaction during follow-up (1 year).

Results

PRP did not improve the outcome of facial lipofilling when looking at skin elasticity improvement, graft volume maintenance in the nasolabial fold or patient satisfaction. Patient recovery after surgery however, dropped significantly. Furthermore, no skin rejuvenation effects from lipofilling could be observed.

Conclusion

This study clearly demonstrates that the addition of PRP to the lipograft significantly reduces patient’s reported recovery time, but does not significantly improve skin elasticity, volume retention nor overall patient satisfaction as compared to lipofilling alone. Moreover, reported effects of ‘normal’ (not SVF/ADSC enriched) lipofilling on skin rejuvenation, as has been reported and suggested to be seen in clinical studies when used in combination with facelift surgery, could also not be addressed.
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Introduction

Lipofilling, i.e. autologous fat transplantation or fat grafting, has become an important treatment modality in facial rejuvenation procedures: it is a safe procedure that requires only limited additional operating time. The presence of ASCs (Adipose stem Cells) in the lipograft could result in tissue regeneration. This has resulted in a paradigm shift towards the combination of facial rejuvenation by using both surgical lifting techniques as well as lipofilling procedures to restore both volume and tissue damage on a cellular level. By this combination of both surgical lifting and lipofilling, effects of gravity, loss of skin elasticity due to elastin degradation, loss of volume due to fat atrophy and bone resorption are all well addressed.

Fat grafting not only restores volume; it is also attributes to regeneration processes that become apparent by improved surface structure and tissue elasticity. Nature’s own regenerative source of wound healing is clot formation after platelet aggregation, homing of the cells involved in repair and fibronogenesis. Another reliable manner to produce injectable clots is the generation of platelet-rich plasma (PRP) and to use that to augment wound healing. PRP both serves as an instant scaffold for regeneration as well as a rich source of pro-regenerative growth factors.

With extensive experience of the use of PRP as an additive to facial lipofilling procedures in our clinic dating back to 2005, retrospective analysis revealed several significant beneficial effects when adding PRP to the lipograft. We hypothesized that the addition of PRP to lipografts would augment tissue regeneration. This hypothesis was subsequently tested in this double-blind randomized placebo-controlled clinical trial for facial lipofilling.

Methods

Study overview

The study conducted was a single-centre, patient and investigator blinded, placebo-controlled trial undertaken at Bergman Clinics The Hague, the Netherlands. A flow-chart overview of the study is shown in Fig. 1. Patients’ follow-up was 12 months in order to obtain long-term lasting results.

The study protocol complied with the Declaration of Helsinki and was approved by local medical ethics committee Zuid-west Holland (National legislator trial code: NL35142.098.11, local METC code: 12-014). All patients provided written informed consent.

Patient population and randomization

Prior to inclusion, a power calculation was performed based on the limited available published data at that time. Following this calculation, aim was to include 32 subjects that would receive facial lipofilling in this study, with one half of the population receiving PRP, the other half a placebo (sterile saline), serving as control group. A detailed description of the randomization process is available online. Inclusion- exclusion criteria were strict, and are listed in Fig. 1.

The primary outcome of the study was skin elasticity improvement (R7 parameter measured by the MPA device) on predetermined fixed measurement locations (see Fig. 2) overlaying the area of intervention. Secondary outcome parameters of the study were: other changes in skin characteristics (R5-R6 parameters, MPA850, same locations), graft take (nasolabial fold decrease) and patient questionnaires regarding recovery time and satisfaction.

Patient enrollment in the study started in 2012 and ended mid 2015. During enrollment in this study, patients were prohibited to undergo further subsequent facial rejuvenating procedures. If a patient still did, the patient was excluded from the study.
**Procedures**

At operating day, but before intervention, measurements were performed by the blinded investigator (JCNW) to determine base line values. All measurements were performed by the same investigator (JCNW) throughout the whole study, for every patient, at every follow-up moment (1 week, 3 months and 1 year post-operative) (Fig. 1) Clear patient instruction was given not to use any skin products on day of the operation nor during the different follow-up moments.

**Figure 1. Study design with a breakdown of enrolled subjects that completed the study, and inclusion/exclusion criteria.**

Excluded: *patient was diagnosed with a gastro-intestinal oncological disease; **patients failed to complete all follow-up moments by not showing up; ‡patient chose to leave the study due to personal circumstances; §patient underwent aesthetic facial surgery during the follow-up.
At the operating theater, with the patient mildly sedated, 30cc of whole blood was drawn from the patient, with an additional 2cc for platelet analysis. Following the pre-trial randomization, opening of the envelope determined whether the whole blood was either discarded, or introduced into the Biomet GPSIII device for PRP isolation (3cc PRP output) following the manufactures protocol. 3cc of sterile saline was used as placebo control. Lipoharvesting, processing and lipofilling were performed following the standard Coleman method: however, both the lipoharvesting - and lipofilling cannulas were significant smaller (harvester: 2.4mm x 22cm, injector: 0.9mm x 5cm). The upper legs served as donor site in all patients. Location and applied lipofilling volume is presented in Fig 3. All procedures have been performed by the same surgeon (HPS), who at that time had already experience with more then 2000 lipofilling procedures. A detailed, step-by-step description of the lipofilling procedure is available online as video supplement.

Figure 2 (left). Locations of skin measurements, shown locations were marked before each measurement. Patients lay down on an examination table to enable correct Cutometer probe placement. Location I: 2cm lateral and 2 cm caudal from the lateral canthus. Locations II: 2 cm lateral from the lateral commissure.

Figure 3 (right). Lipofilling locations and applied volume. Both superficial and deep lipofilling was performed on both sides of the face. In total 18cc per side, 36cc in total. Within the PRP group, 3cc of PRP was added into the lipofilling planes.

Legend:
Deep: Temporal projection (red), Nasojugal groove (green), Central midface (yellow), Nasolabial-fold (blue), Marionette-line, Pre-jowling area and chin (pink).
Superficial: Temporal and central midface area (white), Lower midface-cheek area (black), White rolls (cyan)

Skin measurements
Local skin quality was measured with the Multi Probe Adapter system (C&K Colone Germany) containing the Cutometer MPA580 (elasticity) probe. The cutometer is a valid method in objectifying elasticity of the skin. Measurements were done on fixed locations for every patient (Fig. 2) at every follow-up moment. Before each measurement, the probes were calibrated and tested for correct function. Also local temperature and humidity were logged. True skin elasticity was defined by the Cutometer MPA850 R7 output parameter (the ratio of elastic recovery to the total deformation, elaborated by the R5 (the net elasticity) and R6 (the ratio of viscoelastic to elastic extension) parameters.
**Volumetric changes of the nasolabial fold**

Standardized photographs were captured in three views with a professional 3D camera system (AP, 3Q left and right) at every follow-up moment. Primarily, 3D reconstructions were used to determine volumetric facial changes over time, but were abandoned due to data inconsistency, variation and reproducibility of the measured area. Instead, the pre-operative, 3 months and 1 year post-operative AP-views were used to determine changes in the nasolabial fold depth using a validated grading method (Merz Scale)\(^{17-20}\), that consists of five options (I= minimal fold expression to V= most prominent fold expression). In total, four independent plastic surgeons served as expert panel. The nasolabial fold was chosen because alteration in depth would implicate relevant external changes of facial appearance.

**Patient reported recovery time and satisfaction**

Recovery after the procedure was assessed by means of two patient questionnaires send at 2 and 4 weeks after the operation. Questions included the number of days required to return to work and or resume social activities without using camouflaging agents, and notable changes in facial volume and skin expressed on a visual analogue scale (VAS 1: no changes -10: most significant changes).

![Figure 4](image)

**Figure 4.** Average results: pre-operative (left column), 1 week (center column) and 1 year (right column) AP photographs. Upper row: PRP +, second row: PRP -.

Patient reported satisfaction was recorded by means of a questionnaire send 6 months after surgery: questions included overall satisfaction, changes in volume effect, skin changes and whether or not they would recommend the procedure to a peer (VAS 1-10).
Statistical analysis
Statistical analyses were performed by an independent statistician that received all blinded data by the principal investigator, along with the original randomization from the surgeon. All analyses were done using SPSS 20 (IBM, Chicago, IL, USA). Data figures were generated using Prism 6 (GraphPad Software, La Jolla, CA, USA). The paired samples t-test, ANOVA analysis of covariance and standard linear regression were used. All data fulfilled the requirements for normality and equal variances. A two-sided p<0.05 was considered statistically significant.

Results
In total 32 patients, that met inclusion criteria, were enrolled in this study, with finally 25 patients completing the study. Seven patients were excluded from the study: four patients failed to complete all follow-up moments by not showing up, one patient was diagnosed with a gastro-intestinal oncological disease (ruled as undiagnosed pre-existent, and unconnected with the study ruled by the independent physician), one patient underwent aesthetic facial surgery during the follow-up, and one patient chose to leave the study due to personal circumstances. Excluded patients, unfortunately, could not be replaced due to limited study duration as allowed by the Ethical board.

Of all patients that completed the study, 13 received lipofilling with PRP (PRP+) and 12 lipofilling with saline (placebo, PRP-). Average photographic results are presented in Fig 4. Mean patient age at time of operation was 52 years (±6.75, [38-63]), with no significant age difference between both groups. Whole blood platelet counts were within normal range for all patients (Table 1).

<table>
<thead>
<tr>
<th>Table 1. Study population characteristics</th>
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<tbody>
<tr>
<td>Group I No PRP(n=12)</td>
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<tr>
<td>----------------------</td>
</tr>
<tr>
<td><strong>Mean (SD) [Range]</strong></td>
</tr>
<tr>
<td>Age at time of operation</td>
</tr>
<tr>
<td>Platelet count</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Recorded complications (major and minor)</td>
</tr>
</tbody>
</table>

* students’ t-test
Figure 5. Changes in average true skin elasticity (R7) and R5-6 parameter, for both groups preoperative and during follow-up measured with Cutometer MPA850 at both locations. Data represents group means with SEM. A: R7 parameter location 1 and 2: true skin elasticity, higher values represent an increase in skin elasticity and a positive effect. There is a marginal increase in both groups, with no significance. B: R6 parameter location 1 and 2: the ratio of viscoelastic to elastic extension. Lower values represent a positive effect. C: R5 parameter location 1 and 2: the net elasticity: Higher values represent a positive effect. Again, a minimal gain in both groups, with no significant differences, between both groups or within each group at every follow-up moment.
Lipofilling with or without PRP does not significantly change overlying skin elasticity in this study.

Analyzed R7 parameter data (representing true elasticity) from both groups, showed no significant difference before intervention (Fig. 5). The PRP+ group did not differ significantly from the placebo group at any moment. Data correction for age, room temperature, humidity conditions and baseline (pre-operative) measurements resulted in similar findings.

Analyzed R5 and R6 data showed comparable values in both groups, at every follow-up moment.

Regression analysis of pre-operative R7 parameter as a function of age showed a negative correlation in both groups, comparable to Enzure et al. However, after intervention, the correlation reverses (Fig. 6), which could be a sign of facial rejuvenation. Changes were most noticeable in the PRP+ group: the high prediction value of the regression line (R=0.542, p=0.055) could suggest that sample size in this study was not adequate. Interestingly this reversal was only notable on Location 1 R7, not on location 2 nor with the R5-R6 parameters.

Figure 6. Regression analysis of true skin elasticity as a function of age, before and 12 months after intervention. All measurements and the calculated regression curve with 95% C.I. are presented A: PRP-. Pre-operatively age correlates negatively with true elasticity (y= -0.003293*x+0.4343 R=0.402 p=0.195), but this correlation reverses 12 months post-operatively (y= 0.002005*x+0.1507 R=0.326 p=0.299). B: PRP +. Again, a negative correlation before operation (y= -0.002444*x+0.3471 R=0.392 p=0.184), with a stronger reversal after intervention (y= 0.005078*x+0.01921 R=0.542 p=0.055) compared to PRP -. 
Changes of the nasolabial fold

Summarized data from both groups, at every follow-up moment are presented in Fig. 7, lower scores represent a less prominent nasolabial fold. Grading scores showed a high level of agreement between each expert (all Spearman ICC r >0.576, p<0.001). Pre-operative scores were comparable in both groups (PRP-: μ:2.359 ±0.1531, PRP+: μ:2.622 ±0.2388, p>0.05). Data after 3 months and 1 year also showed comparable results, with no significant differences between both groups at any moment. Furthermore, no changes between pre- and postoperative scores within each group were found.

Figure 7. Result of nasolabial fold grading preoperative and during follow-up. Data represents group means with SEM from grading’s by four experts. Lower scores represent a less prominent nasolabial fold. No significant differences, between both groups or within each group at every follow-up moment could be calculated. A: Pre-operative scores for both groups B: Post-operative scores for both groups C-D: Changes in grading’s during follow-up for both groups. No effect was observed during follow-up.
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### Tabel 2. Recovery time*

<table>
<thead>
<tr>
<th>Facial Lipofilling</th>
<th>Group I PRP- (n=12)</th>
<th>Group II PRP+ (n=13)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td>-RTW/social activities with camouflaging agents</td>
<td>15.4 (9.1)</td>
<td>9.1 (3.7)</td>
</tr>
<tr>
<td>-RTW/social activities without camouflaging agents</td>
<td>20.6 (6.6)</td>
<td>14.9 (4.6)</td>
</tr>
</tbody>
</table>

*Recovery time was defined as the patient reported number of days after surgery returning to work (RTW)/social activities

**Independent sampels t-test

### Addition of PRP speeds up recovery, but does not increase patient satisfaction.

Patient questionnaire reported recovery time, derived from the number of days returning to work/social activities with or without camouflaging agents, showed a significant faster recovery in the PRP+ group (Table 2). Mean number of days returning to work/Social activities with camouflaging agents was 9 days ($\mu=9.133 \sigma=3.701$, $p<0.01$) in the PRP+ group and 15 days in the control group ($\mu=15.43 \sigma=4.949$). Return to work/social activities without camouflaging agents supported this finding (PRP+: $\mu=14.87 \sigma=4.604$ vs PRP-: $\mu=20.57 \sigma=6.61$ $p<0.05$). Questions regarding noticeable differences in facial volume and skin quality after 2 and 4 weeks showed no differences. ($p > 0.05$)

Patient satisfaction, changes in volume and skin quality, reported after 6 months proved to be similar in both groups (data not presented). Overall satisfaction was reported as ‘moderate’. Positive skin changes were reported by several patients in both groups, contradicted by patients that did not notice any skin changes at all. Overall, the level of recommendation of the procedure to peers was negative for both groups, mainly as told by them because of higher expectations of the effect of the procedure.

### Discussion

This randomized placebo controlled double blindered study was undertaken to investigate the possible beneficiary effects of adding PRP to aesthetic facial lipofilling in a well-defined healthy patient cohort. The results clearly demonstrate that the addition of PRP to the lipograft significantly reduces patient’s reported recovery time. However, the addition of PRP to the lipograft does not significantly improve skin elasticity, changing in nasolalial fold depth nor overall patient satisfaction as compared to lipofilling alone. The reversal in the correlation of net elasticity as a function of patient age could suggests some form of rejuvenation by lipofilling that is enhanced by PRP, but lacked significance with the number of patients in this study.

Reported in vitro effects of PRP 10, 22-24 thus could not be reproduced in our clinical study setting, possibly by incontrollable patient related confounding factors combined with a small therapeutic window for effect. Moreover, reported effects of ‘normal’ (not SVF/ASC enriched) lipofilling on skin rejuvenation, as has been reported and suggested to be seen in clinical studies when used in combination with facelift surgery2, 9 could also not be addressed and forces us to question what the additional effect (next to some volume enhancement) of normal lipofilling is when used during facelift surgery.
Lipofilling does not increase skin elasticity in the aging face, even with added PRP.

Since the comeback of lipofilling, suggestions were made that it is ‘more than a filler’ and may induce rejuvenation of the skin. However, this ASC induced effect, is only well studied after deep dermal injury (e.g. thermal-radiation damage, excessive scarring). Surprisingly skin rejuvenation of the normal aging skin has only be described, and studied histologically by Rigotti et al. In this study, an increase in dermal elastin deposition was reported in biopsies after normal lipofilling of the aging skin. However, to this date, no controlled studies were done to verify the clinical relevancy of their finding. In our study, skin elasticity was determined with the Cutometer since it is a reliable and validated method of measuring skin age, and the mostly likely candidate to show changes, supported by the findings of Rigotti et al. Nevertheless, there remains minor controversy regarding the reliability of the Cutometer.

A study of Nedelec et al. presented low intraclass correlation coefficients of skin elasticity measurements of dermal scars. The intraclass correlation coefficients found for normal skin elasticity measurements were, however, acceptable for the R0 (0.81), R6 (0.81) and R7 (0.78) parameter. We found that normal (not SVF/ASC boosted) lipofilling with or without PRP did not alter skin elasticity. Reversal of the correlation between age and elasticity however, might suggest a small effect size, thus not significant with our small study population. Nevertheless, the small effect size raises questions if normal lipofilling is ‘just a filler’ in aesthetic procedures in the aging face which involve only lipofilling. Improvement in outcome when lipofilling is combined with lifting procedures could be explained by the large wound surface created and ASC modulation during healing, downregulating fibrosis pathways. Recent publication on SVF boosted/ASC expanded lipofilling however, do show a significant clinical effect and seem the way forward.

In theory, adding PRP could affect overlying skin true several pathways and cell lines. Angiopoetin-1 and 2, abundantly present in platelets have shown to stimulate endothelial cell growth, migration and differentiation in cultured human dermal microvascular endothelial cells in vitro. Also, PRP-lysate is a strong proliferator for ASC, essential for graft take and a proven down regulator of fibrosis.

Effects of lipofilling with or without PRP on nasolabial fold depth.

Grading of the nasolabial fold during follow-up showed no noticeable lasting effect of lipofilling nor lipofilling with PRP on the depth of the nasolabial fold. Even though the ‘Merz Scale’ used in this study, has shown to successfully differentiate in small volume changes (e.g. filler injection) we could not determine these differences probably because the lipofilling increased overall facial volume, not altering relative differences between facial zones. In our opinion, only in combination with a facelift, lipofilling may additional demonstrate its effect on the nasolabial fold: lifting probably is definitely needed as such. Furthermore, changes in facial volume are minimal because of the limited amount of lipografts that is injected, with uncertainty about the clinical impact of these minor changes if not combined with a lifting procedure. To this date, only one study has been published that reported facial graft retention determined with external 3D photographic reconstruction after aesthetic facial lipofilling. In this study, an overall retention of 32% was reported, however the range and variation of reported data questions its scientific merit. Moreover, the vast number of patients in this study also received some form of lifting procedure that most likely changed distribution of facial volume, and by this means influenced facial volume attributed to lipofilling. Again suggesting that lipofilling should be combined with a lifting procedure in aesthetic facial rejuvenation. Even though lipograft survival in the face has been documented with MRI imaging, the clinical relevancy of aesthetic facial lipofilling procedures without lifting procedures on facial fold depth remains to be determined.
With ongoing uncertainty about lipograft survival, several fundamental studies explored PRP addition and found positive effects. Graft take might improve by PRP effects on ASC proliferation, blockage of apoptosis pathways and differentiation into adipocytes. Moreover, PRP lysate stimulates proliferation, migration and tube formation of human umbilical vein endothelial cells both in vitro as well as in a nude mouse model. PRP induces changes on endothelial cells that can contribute to (neo)angiogenesis of the fat graft and thereby enhance fat graft survival. These findings however, fail to make a significant impact in the majority of available clinical PRP-lipofilling studies, thus questioning clinical use of PRP addition to lipofilling for this reason.

**PRP speeds up patient recovery**

Patient reported recovery time was significantly reduced by the addition of PRP in this study. This finding is in line with previous data from our retrospective study and current literature on aesthetic procedures like fractional carbon dioxide laser resurfacing treatment. Dermal- and wound closure effects observed after PRP injection might be explained by the effect from PRP on fibroblasts. In vitro study of Ramos-Torrecillas et al. eases the growth of fibroblasts and induces their differentiation into myofibroblasts, thus playing a key part in wound contracture. Collagen 1 and extracellular matrix remodeling by fibroblast is also affected by PRP. Fibroblast exposed to PRP lysate in vitro up regulates the expression of Matrix metalloproteinase (MMP)-1, which in its turn plays a key role in collagen remodeling. Also, type 1 collagen expression is increased under these circumstances. Increased fibroblast activity, along with changes in collagen production and a potentially stronger inflammation response could also play a role in our observed reduced recovery time after surgery when the lipograft was combined with PRP.

**The concentration paradox: Less is more?**

A potential pitfall in evaluating the effect of PRP is the lack of uniform concentrations of created PRP. The studies of Yamaguchi et al. were the first publications that showed that a higher concentration of PRP (or more platelets) may produce counterproductive effects, possibly by unwanted cell differentiation. Most commercially available PRP kits capture a percentage of available platelets from whole blood, not a certain quantitative number of platelets. Considering the fact that normal human platelet counts are defined within a wide range and show large daily variations, the cumulative amount of growth factors in kit-isolated PRP is inconsistent. This variation can inadvertently influence its effect in a way as is observed in vitro on different cell types. Regarding cells present in the lipograft, PRP concentration alters ASC proliferation, function and behavior. High PRP concentrations increase proliferation, but also changes ASC into a fibroblast like phenotype, with increased collagen RNA expression and altered paracrine signaling that negatively influences endothelial vessel formation. Although platelet counts were normal within our well-defined healthy patient cohort, combined with comparable fat-graft-PRP-or-placebo mixture ratios, our study is potentially biased and weakened by this concentration-depended effect. Moreover, this phenomenon could explain the failure of clinical studies.

Local growth factor conditions after lipofilling are also an issue that remains unclear; in a healthy patient, the release of platelets and pro-inflammatory factors due to damage caused by the lipofilling procedure itself could be of such an extent that the addition of PRP actually is insignificant and/or redundant or even too high.
Conclusion

This randomized double-blinded, placebo-controlled study clearly has shown that PRP significantly reduces post-operative recovery time but does not improve patient outcome when looking at skin elasticity, improvement of the nasolabial fold nor patient satisfaction. The reversal of the correlation between age and elasticity might indicate for some effect on skin, but requires more power of future studies.

Thus far, the use of PRP as an additive in lipofilling has shown great promises in vitro. These beneficiary effects, however, have only partially been reproduced in a clinical setting. A growing number of studies report a concentration depended effect of PRP in vitro, making optimal use in a clinical setting delicate and complex. Further studies of PRP interactions on both the lipograft as well as the receptor host site involved cells seems to be of paramount importance to determine the optimal use and concentrations of PRP in a clinical setting.
References

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