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ORIGINAL CONTRIBUTION

Lower facial regeneration with a combination of platelet-rich fibrin liquid matrices based on the low speed centrifugation concept-Cleopatra technique

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Abstract

Background: Autologous blood concentrates are increasingly being applied in esthetic medicine and dentistry due to their safety and their potential beneficial properties. Platelet-rich fibrin based on the low speed centrifugation, a newly described blood product, seems to convey additional properties in several in vitro and ex vivo studies. Its clinical significance however in relation to facial regeneration remains anecdotal.

Objective: The aim of this study was to assess a specific combination of PRF liquid matrices utilized for lower facial regeneration (Cleopatra technique).

Patients/Methods: Cleopatra technique was applied in 32 patients. In addition to recording of all patients' complaints and adverse events, a photographical study was performed. Possible positive effects were assessed by asking twenty-three independent reviewers to distinguish initial and later photographs of each patient.

Results: A statistically significant percentage of true answers by the reviewers was noted upon completion of the treatment ($U = 110.5$, $P < .001$), which indicates a clinically significant effect of Cleopatra technique. Moreover, only few minor, self-limited adverse events were recorded.

Conclusions: Cleopatra technique is a well-tolerated and effective method of lower facial rejuvenation that deserves further attention from dentists and other health professional who utilize conservative methods in facial esthetics.

KEYWORDS

facial regeneration, PRF liquid matrices

1 | INTRODUCTION

1.1 | The aging face and lower facial regeneration

Facial manifestations of aging reflect the combined effects of gravity, progressive bone resorption, decreased tissue elasticity, and redistribution of subcutaneous fullness. Several effects can be noted in the lower third of the face because of these changes, such as a relative excess of skin, a loss of definition of the jawline, formation of facial jowls, protrusion of the central chin, the characteristic "turkey neck" deformity, the creation of horizontal rhytides, and many others.[1] The rejuvenation procedures aim at restoring the ample, balanced distribution of facial fullness that exemplifies the youthful face.[2] A significant increase in the number of nonsurgical procedures performed for facial rejuvenation can be seen lately due to their advantages of an immediate cosmetic result and a short recovery time.

1.2 | Platelet concentrates

Over the last decade, the use of autologous blood concentrates, such as platelet-rich plasma (PRP) and platelet-rich fibrin (PRF), has gained importance in esthetic medicine for dermal stimulation, augmentation, and rejuvenation.[3-6] There are numerous studies (observational, in vitro, animal models, and clinical trials) suggesting a tangible effect of both topical and injectable applications of platelet concentrates on cellular changes and facial regeneration.[7]

This is mostly because facial regeneration utilizing autologous platelet growth factors is considered a natural approach to restore dermal degeneration in contrast to exogenous growth factors and biodegradable substances. Moreover, platelets preparations, apart from their bulking effects as fillers, release numerous growth factors, cytokines, and extracellular matrix proteins upon their activation, such as fibrin, fibronectin, and vitronectin, that bind to their specific cellular receptors and enhance or modify the various intracellular processes that relate to cell proliferation and production of additional extracellular matrix proteins.[8]

1.3 | PRP and PRF

Platelet-rich fibrin and PRP are the most commonly employed platelet preparations. Platelet-rich plasma, considered the first generation of platelet concentrates, includes mainly platelets and plasma proteins. Its preparation procedure consists of two-step centrifugation and requires the addition of external anticoagulants. In addition, xenogeneic thrombin or calcium ions are added to PRP to activate platelets to release growth factors.[5] The use of external chemicals and activation factors may enhance the contamination risk and make the use of PRP in clinical routine an elaborate procedure. The further development of platelet concentrates led to the introduction of PRF, which is a fully autologous system. Platelet-rich fibrin is obtained through one-step centrifugation without any anticoagulants.[6] In addition to fibrin, platelets, and plasma proteins, PRF includes a high number of leukocytes.[7] Depending on the blood-collection tube and the centrifugation protocol, it is possible to generate either a solid or a liquid PRF matrix without anticoagulants. In terms of solid PRF, platelets interact with the glass surface of the tube and activate their coagulation during the centrifugation procedure.[2,6] Immediately after centrifugation, the resulting solid PRF matrix consists of a fibrin scaffold with entrapped platelets, leukocytes, plasma proteins, and growth factors. The liquid PRF is generated using a blood-collection tube with a plastic surface that enables the generation of a liquid PRF matrix without the use of external anticoagulants. The resultant liquid PRF preserves its liquid condition for approximately 15-20 minutes and forms a fibrin clot thereafter.[8]

1.4 | The low speed centrifugation concept (LSCC)

Extensive research aiming to understand the effects that centrifugation has on the components and bioactivity of PRF introduced the so-called low speed centrifugation concept (LSCC).[2] This concept states that reducing the applied relative centrifugation force (RCF) during the centrifugation of PRF matrices significantly enhances the platelet and leukocyte number in the resultant PRF matrix. Additionally, PRF matrices that are prepared using a low RCF release significantly higher concentrations of key growth factors, such as vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), and platelet-derived growth factor (PDGF-BB), compared with matrices prepared with a high RCF[2,8-10] and seem to convey greater regenerative potential than previous platelets preparations.[9]

Several platelet-rich fibrin matrices can be produced based on the LSCC. A systematic evaluation of the influence of the relative centrifugal force on cell types and growth factor release within these matrices has been performed[10] that have led to the characterization of mainly two types of matrices performed by two different low speed centrifugation protocols:

1. Lower RCF protocol: 3 minutes at 700 rpm (60 *g*) for women and 4 minutes at 700 rpm for men.
2. Higher RCF protocol: 5 minutes at 1300 rpm (208 *g*).

Apart from the statistically significant differences that have been observed in relation to inflammatory cells and platelets counts as well as to the release of various growth factors, these protocols also lead to PRF matrices of different volumes and mechanical properties. More specifically, although PRF matrices produced by higher RCF have less platelets, white cells, and growth factors, their volumes are about 3 times greater since they contain more fibrin and other plasma proteins that are needed for volumetric purposes (structural support and filling effects). Thus, a rational hypothesis is that a combination of PRF matrices produced by the LSCC can be beneficial, since they convey different and potentially useful properties. Herein, we present a new technique (Cleopatra technique) based on this combination, that can be easily applied by dentists and other health professionals who are familiar with platelet concentrates, either alone or in combination with other treatments (eg, denture work, superficial skin rejuvenation with other conservative treatments, and full face rejuvenation). Its name comes from the first name of the author (initials deleted as required by the journal for paper submission) who applied and first presented this technique in various conferences and in a book chapter (reference deleted for initial paper submission since it reveals the name of the first author).[11] Initial outcomes based on satisfaction score, photographic materials, and possible complications are also presented.

2 | MATERIALS AND METHODS

Thirty-two subjects offered lower facial rejuvenation, either alone or in combination with other esthetic interventions, were included in the study. An ethical approval was granted by the Ethical and Scientific Committee of the Plastic and Reconstructive Surgery Department of Agioi Anargyroi General Oncological Hospital of Kifisia, Athens, Greece (No. 1/26/09/2016). All patients signed an

informed consent prior to their treatment. The subjects were otherwise healthy women with a mean age of 48 (25-65 years old).

2.1 | Cleopatra technique

All patients were treated by the author (CN) using a standard protocol (Cleopatra technique). More specifically, a total of 4 sessions of PRF treatments were offered with 2- to 3-week intervals between each session. At the beginning of each session, 60 mL of venous blood was collected in 10 mL PRF tubes (Orange Plastic tubes, Process for PRF, Nice, France) and centrifuged according to the aforementioned low speed centrifugation protocols:

1. Lower RCF protocol: 3 minutes at 700 rpm (60 *g*). Thirty ml (3 tubes) was utilized that resulted in 3-4.5 mL of PRF liquid, according to each individual (reddish color due to a relatively increased number of cells).
2. Higher RCF protocol: 5 minutes at 1300 rpm (208 *g*). Thirty ml (3 tubes) was utilized that resulted in 7.5-9 mL of PRF liquid (yellowish color due to more fibrin).

A preprogrammed centrifuge with a radius of 110 mm (Process for PRF, Nice France) was used. A total of 10.5-13.5 mL of PRF was produced by the two abovementioned centrifugation protocols that were mixed within the same syringes. Total maximum volumes of PRF injected in the areas of the lower face bilaterally are shown in Table 1.

Volumes may differ depending on the skin conditions, for example, relatively greater endodermal injections in thick, dehydrated older faces. In the great majority of cases, 10.5-12 mL of a PRF mixture produced by the abovementioned protocols was sufficient for the Cleopatra technique. In cases where greater volumes may needed, more blood can be collected and centrifuged using these protocols.

In more details regarding injection technique, micro-papular or serial puncturing technique was used for endodermal or superficial subdermal injections with a 32-30 g, 4 mm syringe. For more deep injections 30 g, 12.8 mm or 27 g, 12.8 mm syringes were utilized. For those injections, a linear threading or retro-tracking technique was used mainly for smokers' wrinkles. Fan technique (utilizes the same skin entry point) is mainly for restoring and creating volume, for example, for marionette lines, whereas sandwich technique can be used when further volumizing is needed, for example, in nasolabial folds. Bolus PRF injections were avoided, as well as massage after treatment.

TABLE 1 Total maximum volumes of PRF injected in the areas of the lower face bilaterally

Nasolabial folds 6 mL (3 mL each side)	Marionette lines 2 mL
Oral commissures 2 mL	Mandible and prejowl sulcus 2 mL

2.2 | Pre- and post-treatment preparations

Exclusion criteria are related to contraindications for blood concentrates and can be seen in Table 2.

Upon the informed consent and the appropriate photographic recording, a thorough cleaning of the skin, with an alcoholic solution, was performed and topical anesthetic (EMLA 5%) was applied. Infiltration with local anesthetic was used in the majority of cases. Subjects were instructed to avoid alcohol consumption, aspirin, NSAIDs, warfarin, and vitamin E a few days before each session. Those who were prone to herpes simplex, acyclovir tablets were recommended 2 days prior to the treatment and another 4 days after the treatment, whereas a melanin inhibiting skin regime for at least 2 weeks prior to treatment was also prescribed for tanned to dark complexions or skins prone to postinflammatory hyper-pigmentation. The patients should not have any facial treatments at least 30 days before the treatment such as chemical peels and fractional laser which damage the skin.

Upon completion of each treatment, a face mask with hyaluronic acid and vitamins was applied on the face for 15-30 minutes according to the skin condition. For home care, a moisturized ointment, ice, or Arnica Montana was prescribed according to the needs of the skin. The patient was informed about the necessity of a sun protection cream with at least 30% SPF in order to protect the skin from sun damage.

2.3 | Assessment

One frontal and two oblique views of the lower face were captured before each session as well as about 2 weeks after the completion of the treatment (Figure 1). Twenty-three independent (blinded) reviewers were asked to assess the initial photographs and those captured before the second session and to put them in chronological order. The process was repeated with those photographs captured at the end of the treatment. Photographic material from patients offered additional treatments such as dental prosthesis that can bias the evaluation was excluded from the study. In addition, photographs with obvious signs of make-up that could affect the assessment were also excluded. Percentages of right answers were calculated for each reviewer (rater). Mann-Whitney *U* test was used to compare the percentages of right answers between the two assessments.

TABLE 2 Contraindications/exclusion criteria for Cleopatra technique

CONTRAINDICATIONS-exclusion criteria
Anaphylaxis or history of multiple severe allergic reaction
Compromised immunity due to disease or treatment (eg, corticosteroids and cytotoxic drugs)
Chronic disease such as cancer and renal failure
Severe anemia or conditions that lead to poor platelet count
Pregnancy and breast-feeding

FIGURE 1 Frontal and oblique views of the lower face captured before the first (A), the second (B), and 2 wk after the completion of the treatment (C)

TABLE 3 Comparison of the percentages of true answers between the two photographic assessments

	1st photographic assessment: initial-prior to 2nd session	2nd photographic assessment: initial-after completion of the treatment	*U*-score, *P*-value
Mean percentage of true answers (25%-75%)	47.07 (43-52)	60.11 (52-70)	110.5, <.001

3 | RESULTS

Platelet-rich fibrin rejuvenation with the Cleopatra technique was performed in 32 patients. Nine of them were excluded from the photographic study due to additional dental works or visible signs of make-up that could affect lower facial esthetics.

Mean percentages of true answers of the blinded ratters regarding the order of the photographs (initial-prior to 2nd session as well as initial-after completion of the treatment) are seen in Table 3. A statistically significant difference was found between the two comparisons indicating a visible effect of Cleopatra technique upon completion of treatment.

In more details, as it can be seen in Table 3 reviewers failed to place the photographs in the right chronological order almost in the majority of cases when assessments were made before the initiation of the treatment and just prior to the 2nd session. Percentage of true answers, however, differed significantly upon completion of the treatment (U110.5, $P < .001$), with the majority of reviewers finding the right chronological order.

Regarding adverse events, 5 patients complained of mild pain during the procedure that was alleviated with a slower rate of injections apart from 2 of them who required paracetamol p.o. for pain relief. One patient developed severe redness immediately upon completion of the treatment that subsided within 30 minutes, and two others complained of bruises that treated with arnica ointment.

4 | DISCUSSION

Facial esthetics of the perioral area (cheeks, nasolabial folds, lips, marionette lines, contouring of the face) are increasingly performed by dentists in the majority of countries. This reflects a trend toward more conservative procedures or alternatives to surgical restoration. This is rational since enhancement of facial features, correction of facial asymmetry, and restoration of facial volume that has been lost due to aging cannot be achieved with the conventional excision and suspension procedures. Natural filling materials, such as collagen, hyaluronic acid, fat, PRP, and PRF, are a logical and possibly effective treatment choice for this purpose, either alone or as an adjunct to surgical facial rejuvenation techniques.

Autologous sources for soft tissue augmentation have been sought by physicians, especially in the field of esthetic medicine

due to the possible transient effects associated with foreign materials including the possibility of granuloma formation and chronic or delayed infections caused by biodegradable foreign substances.[12] Furthermore, exogenous autologous growth factor safety, especially in relation to cancer, has not been established. As a result, autologous platelet products containing growth factors such as PDGF, transforming-growth factor (TGF, especially TGF-b), VEGF, insulin-like growth factor (IGF), and EGF have been studied extensively over the last decade.[13] Apart from their direct bulking effects, platelet concentrates promote angiogenesis and cause an enhanced production of collagen and fibronectin.[14] They have been shown to retain their chemotactic and/or mitogenic action for various cells including monocytes, fibroblasts, stem cells, smooth muscle cells, endothelial cells, and keratinocytes.[15] Briefly, blood concentrates are increasingly being utilized as autologous products for esthetic purposes because they contain platelets for growth factor release, fibrin scaffold for tissue remodeling, plasma proteins for collagen synthesis, white cells for inflammation and recovery, and stem cells for tissue repair and regeneration.

In addition, an in vivo study showed that optimized PRF matrices prepared according to LSCC provide significantly higher vascularization than do PRF matrices that are prepared using a high centrifugation force[16] and seem to convey greater regenerative potential than previous platelets preparations.[9] Therefore, liquid PRF can serve as an autologous source of growth factors that triggers cell-mediated vascularization and supports regeneration. Moreover, genetic factors could be utilized as biomarkers or predictors for regenerative protocols with PRF in the field of facial esthetics.[17]

To our knowledge, this is the first published study to show a clinically significant benefit of liquid PRF matrices as a single treatment modality on facial regeneration. Furthermore, as it is expected due to their autologous nature, these matrices are relatively safe with minimal and self-limited adverse effects. Although further studies with the inclusion of larger number of patients may be difficult to be organized, especially in a private setting, these are certainly of interest since they can provide more robust evidence on the effectiveness of Cleopatra technique. Possibly, a multicentre study design could overcome this obstacle. In addition, more long-term follow-ups are required in order to conclude for the duration of the effect. Nevertheless, Cleopatra technique is a safe, easy to perform, well-tolerated technique that deserves further attention from dentists and other health professional who utilize conservative methods in facial esthetics either as a single base modality or in combination with other techniques.

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