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Pilot study: Autologous platelet-rich plasma used in a topical cream for facial rejuvenation

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Abstract

Background: Platelet rich plasma (PRP) is traditionally used as an injectable material for enhanced healing, hair growth, and facial rejuvenation.

Aims: This research examined the novel use of topical autologously sourced PRP added to a preservative cosmetic base and applied twice daily to the face following electroporation for 8 weeks.

Methods: 20 healthy female and male subjects 30-60 years of age were enrolled in this single-site, investigator blinded, vehicle controlled split-face study to evaluate the effect of a PRP-containing serum versus the serum alone on facial photoaging.

Results: 90 day stability for the PRP in a preservative serum was achieved with refrigeration at 4 degrees Celsius. Facial skin biopsy histologic findings included improved rete peg architecture. Immunohistochemical analysis showed upregulation for collagen type I with qPCR data demonstrating concomitant upregulation of mRNA for collagen after 8 weeks of topical PRP use.

Conclusion: These pilot study findings may indicate value for topical PRP in facial rejuvenation.

KEYWORDS

aging skin, cosmetics, facial rejuvenation, platelet-rich plasma, PRP

1 | INTRODUCTION

The basic human need to look young has led to many concepts in facial rejuvenation. One such concept is the adaptation of platelet-rich plasma (PRP) for dermatologic uses.[1] PRP was originally developed to improve healing in orthopedic and dental surgery, but is now used in plastic surgery via injection for wound healing and hair growth.[2,3] The next horizon is the adaptation of PRP for topical uses in cosmetic formulations.

Platelets are composed of a cytoskeleton and intracellular structures such as glycogen, lysosomes, and two granules, the dense granule and the alpha-granule. The granules are the most valuable structures for the development of the current topical product. The dense granule has adenosine diphosphate (ADP), adenosine triphosphate (ATP), serotonin, and calcium, while the alpha-granule contains clotting factors, growth factors, and proteins.[4] The main goal for the topical product is to keep the enclosed cellular components active throughout the intended time period of use and obtain enough platelets to be considered platelet-rich plasma.

The normal platelet count in human blood is 150 000-350 000/microliter. PRP is defined as 1 000 000 platelets per microliter in a small volume of plasma with a full complement of clotting factors. There are seven key growth factors in PRP: platelet-derived growth factors (PDGFaa, PDGFbb, PDGFab), transforming growth factors (TGF-b1, TGFb2), vascular endothelial growth factor (VEGF), and epithelial growth factor (EGF).[5] These growth factors are found within a normal clot composed of fibrin, fibronectin, and vitronectin, which are cell adhesion molecules required for cell migration, as seen in wound healing.[6] Cellular mitogenesis and angiogenesis are both upregulated by PRP, making it useful in facial rejuvenation.[7]

Autologous PRP is obtained from freshly drawn blood from the patient with an added anticoagulant and the sample then experiences a series of two centrifugation (spin) steps.[8] The first spin, known as the hard spin, separates the red blood cells from the plasma containing the platelets, white blood cells, and clotting factors. Three layers result from the hard spin: an upper layer containing platelets and white blood cells, a middle layer known as the buffy coat containing white blood cells, and a bottom layer containing red blood cells. The red blood cell layer is removed and discarded. The second spin, known as the soft spin, separates the platelet-rich plasma in the bottom of the tube from the platelet-poor plasma (PPP) in the top of the tube by removing more red blood cells.[9] Proper preparation and centrifuge technique is critical to obtaining high quality active PRP.[10] The literature has pointed to some preparations of PRP having a lack of biological effect which may be due to poor PRP processing or inadequate standard laboratory centrifuges that cannot properly prepare PRP rather than the specialized FDA cleared equipment with validated processes.[4]

Platelet-rich plasma is autologous, thus concerns about immune rejection are a nonissue.[11] Growth factors function by activating a cytoplasmic signal that promotes normal gene expression. PRP contains the same materials present in the blood stream that induce clotting, except in higher concentration.[12]

Platelet-rich plasma works through degranulation of the alpha granules in platelets, which contain the growth factors. It is ideal to collect PRP in an anticoagulated state for the growth factors to remain active explaining the need to draw the blood into a tube containing sodium citrate.[13] The biologically active cellular components such as the growth factors as preserved within the platelet when the platelet is held in an inactive state; premature degranulation does not occur, and therefore the cellular components are held within the protective enclosure of the platelet.[14] Upon activation of the platelet, the granules then fuse to the cell membrane, the degranulation process, activating the secretory growth factors, which bind to the transmembrane receptors of target cells, such as mesenchymal stem cells, fibroblasts, endothelial cells, and epidermal cells.[15] This binding activates intracellular signal proteins that express a gene sequence directing cellular proliferation, collagen synthesis, extracellular matrix formation, and numerous other pathways to promote healing and repair processes.[16] Damaged platelets with degraded or not viable cellular components are incapable of inducing this response.[17]

The current pilot study examined the use of an autologously sourced PRP added to a preservative cosmetic base and applied twice daily to the face following electroporation for 8 weeks. Visual, photographic, histologic, immunohistochemical, and molecular qPCR data were obtained to better understand the role of topical-administered PRP in facial rejuvenation.

2 | METHODS

Twenty (20) healthy female and male subjects 30-60 years of age were enrolled in this single-site, investigator blinded, vehicle-controlled split-face study to evaluate the effect of a PRP-containing serum on facial photoaging. Subjects who signed consent and met all inclusion criteria and none of the exclusion criteria were enrolled at the baseline visit (Allendale Institutional Review Board, Old Lyme CT). Subjects were asked to continue their self-selected cleanser and nonmedicated facial cosmetics throughout the 8-week study. Subjects were randomized to apply the PRP-containing serum to the right or left face and the serum alone to the opposite side of the face.

Dermatologist investigator and subject assessments for efficacy and tolerability were conducted on a 5-point ordinal scale (0 = none, 1 = minimal, 2 = mild, 3 = moderate, and 4 = severe) separately for each cheek. The following efficacy parameters were assessed: dryness, lack of tactile smoothness, lack of visual smoothness, lack of softness, lack of luminosity, lack of radiance, lack of firmness, poor skin texture, fine facial lines, wrinkles, poor skin tone, mottled hyperpigmentation, and overall appearance. Tolerability was assessed in terms of itching, stinging, burning, redness, and swelling. Transepidermal water loss (TEWL) was performed from the right and left cheeks (Cyberderm, Broomall, PA) and Visia CR 4.3 (Canfield Scientific) photography of the front, right, and left sides of the face were completed before any treatments as baseline documentation. All these activities were repeated at week 4 and week 8.

Subjects underwent phlebotomy to harvest 50ml of blood drawn into a tube containing 10 mL of anticoagulant (EmCyte Corporation), which underwent double centrifugation (EmCyte Executive Series Pure PRP Centrifuge, EmCyte Corporation) to yield 6-9 mL of PRP. The PRP was added to the serum and applied to one randomized side of the face with the opposite side receiving the serum alone. To enhance penetration of the PRP, subjects underwent electroporation with a hand-held electroporation device (RUMI, SheNB Ltd) for 5 minutes to the cheeks, forehead, and periocular area. The study subjects were instructed to keep test articles refrigerated at all times. Subjects were dispensed a sunscreen containing SPF30 moisturizer (Eucerin Daily Protection Face Lotion, Beiersdorf) and a compliance diary.

A subset of 4 subjects participated in the biopsy sub-study undergoing a 2 mm punch biopsy from the left and right preauricular areas, following anesthesia with 2% lidocaine plus epinephrine. The specimens were processed, sectioned, and hematoxylin and eosin (H&E) stained. H&E stained 5 μm serial sections from paraffin blocks and were digitally scanned (NanoZoommer, Hamamatsu). Length profile measurements of the stratum basale and stratum granulosum layers of the epidermis were obtained, generating a ratio between basale/granulosum layers. Nuclear counting and epithelial thickness measurements were conducted. Quantitative immunohistochemistry analysis for collagen I, and elastin were quantified using a color deconvolution algorithm from digitally scanned immunohistochemistry slides. Quantitative polymerase chain reaction was conducted analyzing Collagen 1A1 (COL1 A), keratinocyte proline rich protein (KPRP), and matrix metalloproteinase 1 (MMP1) genes.

Quantitative PCR (qPCR) was performed on biopsy tissue samples that were preserved in RNA later solution. Fold change in mRNA

expression between treatment and control for each subject was calculated using the following equation:

$$\text{Fold Change (FC)} = 2^{-(\Delta\Delta C_T)}$$

An average fold change of 2 represents a significant upregulation in gene expression, and a 0.5-fold change represents a twofold decrease (downregulation) in gene expression.

Next, the opposite integer of the $\Delta\Delta C_T$ value was determined.

$$-\Delta\Delta C_T = \log_2 (\text{FC})$$

Lastly, an average FC was calculated for the specific probe analysis according to the following formula:

Average $\text{FC} = 2^{\bar{x}}$, where $\bar{x}$ is the average of the $-\Delta\Delta C_T$ values.

Ordinal investigator and subject nonparametric data were analyzed using a Mann- Whitney paired two-tailed analysis evaluating change from baseline comparing the active PRP + serum-treated side of the face to the serum-only treated side of the face. The parametric TEWL, histology, and immunohistochemistry data were analyzed using a paired Student's *t* test ($P \leq .05$).

3 | RESULTS

All 20 subjects in the main study and 4/4 subjects in the biopsy substudy successfully completed the study. No tolerability issues were noted by either the blinded investigator or the subjects with either the PRP + serum or the serum alone. This was confirmed by the lack of change in TEWL readings from both sides of the face. In addition, no statistically significant differences between the PRP + serum treatment or the serum alone treatment were noted by either the blinded investigator or the subjects after 8 weeks of use in any of the assessed parameters. Both sides of the face demonstrated statistically significant ($P < .001$) improvement for dryness, tactile smoothness, visual smoothness, softness, luminosity, and radiance at 8 weeks. PRP + serum did demonstrate directional improvement of radiance, luminosity, smoothness at 4 weeks, increasing at 8 weeks vs the serum alone.

The histopathology findings demonstrated a qualitative improvement in the PRP + serum- treated group with a trend of greater rete peg presence in the PRP + serum group compared with base serum controls (Figure 1). Immunohistochemistry revealed enhanced Collagen type I expression in the serum + PRP treatment versus the serum without PRP (Figure 2). These collagen findings were further supported by the qPCR data. Three target genes (collagen IA, matrix metalloproteinase 1 gene, and keratinocyte proline rich protein) and a housekeeping gene control was evaluated. Results demonstrated a 2.34-fold increase in collagen gene upregulation in the PRP treatment serum vs. control serum for collagen type I (Table 1). Samples form subjects 2 and 4, produced samples that did not produce threshold counts; consequently, a fold change could not be determined.

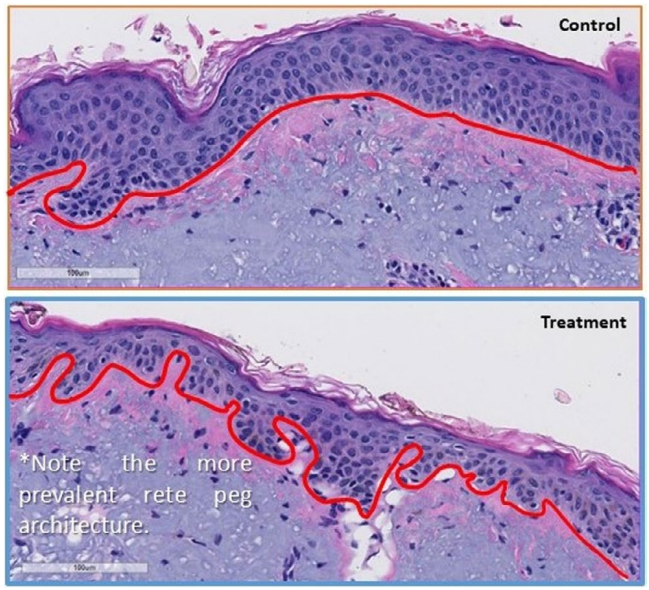

FIGURE 1 Rete Peg Structure. Improved rete peg architecture was observed post topical PRP treatment

The PRP + serum formulation was evaluated for PRP stability as the effective duration of blood-derived products has always been a limiting factor for topical application (Figure 3). Intact PDGF was identified in the 4 degree Celsius constantly refrigerated preservative serum 90 days after preparation. This was an important finding to allow further development of topical PRP cosmetics. Additional studies are underway, including a follow-on publication detailing long-term platelet stability.

4 | DISCUSSION

There remains a paucity of controlled, randomized, blinded studies to evaluate the efficacy of topically applied PRP products for facial rejuvenation. This study was intended to address this knowledge gap. One of the biggest challenges in topical PRP use is the need to stabilize the blood product in a vehicle that provides excellent esthetic properties. Part of this project was to develop a serum suitable for PRP use with an appropriate tolerability profile. No tolerability issues arose and the serum demonstrated 90-day stability. A traditional cosmetic serum is not suitable for use with PRP because commonly used preservatives will destroy the platelets. The study serum contained the preservatives honeysuckle extract and O-cymen-5-OL, which is an antifungal. In addition, the PRP requires a nutrient source and proper buffer system to maintain pH. Glucose, sodium chloride, sodium citrate, sodium acetate, sodium bicarbonate, potassium phosphate, potassium chloride, and magnesium chloride fulfilled this need. Finally, a film-forming agent is necessary to keep the PRP on the skin, and polyacrylate crosspolymer-6 met this need because of its stability at high pH.

The subjects applied the PRP + serum twice daily for 8 weeks. It appears this application period was insufficient to achieve

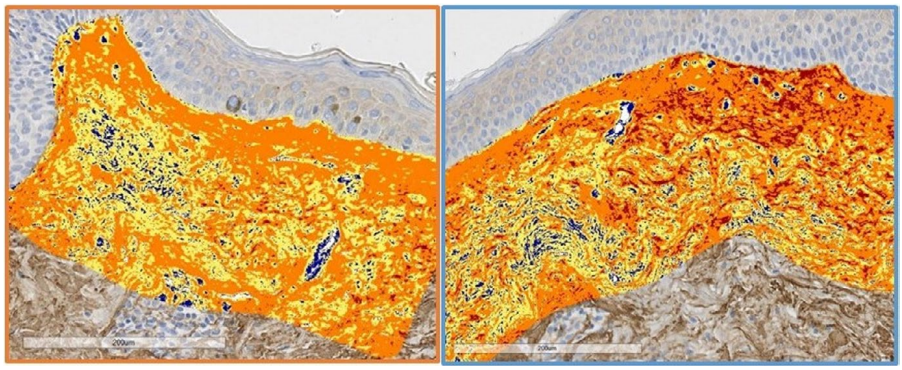

FIGURE 2 Collagen I Immunohistochemistry. Increased signaling for collagen I was observed post topical PRP treatment

TABLE 1 Summary of Q-PCR Analysis

Summary of Q-PCR Analysis			
	Col-1A (FC)	KPRP (FC)	MMP-1 (FC)
Subject 1	1.31	0.83	0.227
Subject 2	8.53		
Subject 3	1.87	1.03	9.39
Subject 4	1.44	1.09	
Average (FC)	**2.34**	0.98	1.45

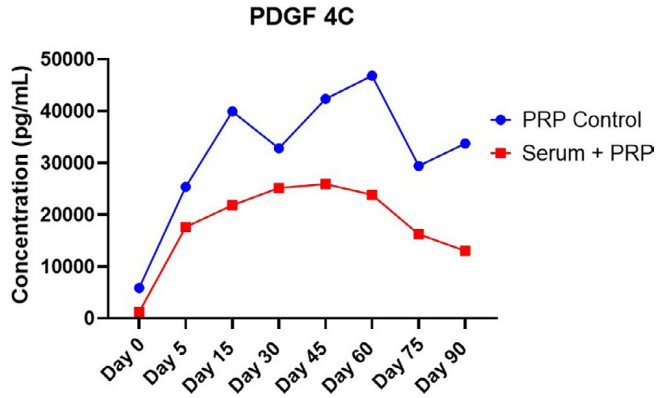

FIGURE 3 PRP Stability. 90-day platelet-rich plasma stability is demonstrated for platelet-derived growth factor refrigerated at 4°C

statistically significant observed results by the investigator and subjects. The PRP + serum did demonstrate directional enhanced performance at 4 weeks, increasing in performance at 8 weeks for radiance, luminosity, firmness, and softness vs the base serum alone. Further, the PRP + serum demonstrated less epidermal cell compacting and greater cellular hydration vs the serum alone. In addition, rete pegs typically extend into the dermis in younger individuals helping to solidify the integrity of dermal-epidermal junction (DEJ), improving skin strength. With increasing age, the rete pegs regress into the upper epidermis. The PRP + serum demonstrated continued elongation of the rete pegs versus the serum alone, with retraction of the rete pegs into the upper regions of the epidermis. Less rete peg connectivity to the DEJ results in the fragile crepey skin observed with aging. Immunohistochemistry results demonstrated higher levels of collagen type I and qPCR results showed upregulation of collagen mRNA. These are promising early observations pointing for the need to increase the clinical exposure of the subjects to the PRP + serum by extending the study period.

Another challenge for topical PRP products is the penetration of the proteins into the epidermis, since the epidermis is uniquely designed to minimize protein penetration. This challenge was overcome using a hand-held electroporation device, which applied an electromagnetic field to the skin surface in order to increase permeability.

This research successfully addressed several of the topical PRP product challenges to include delivery and penetration; however, more research is needed to obtain a better idea of the duration of application required for optimal clinical benefits. PRP has proven to be an effective additive therapy for the healing of tendons, dental bone grafts, facial cosmetic surgery, and diabetic ulcers.[18-20] Its utility in dermatology should be promising, but more research is needed to overcome the challenging attributes of topical applications.[21] Topical PRP treatment may have translational applications in post-procedure applications for laser resurfacing, microneedling, radiofrequency tissue tightening, and dermabrasion.

5 | SUMMARY

Stability of PRP in a preservative serum was documented in this pilot study paving the way for future topical application research. Histologic findings attributable to 8 weeks of PRP + serum topical application included improved rete peg architecture. Immunohistochemical analysis showed upregulation for collagen type I with qPCR data demonstrating concomitant upregulation of mRNA for collagen after 8 weeks of use. Studies are currently underway, considering the positive stability findings, to extend the use period to 16 weeks to determine if early histologic and immunohistochemical and molecular findings will translate into clinical efficacy. The combination of an esthetic procedure followed by topical PRP may provide a new, innovative approach in facial rejuvenation.

CONFLICT OF INTEREST

Zoe Diana Draelos, MD, and Robert S. Kellar, PhD, received grants from Aesthetics Biomedical to conduct this research. Lawrence A.

Rheins, PhD, and Shaun Wootten, BSE, are employees of Aesthetics Biomedical, Inc

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