# Platelet-rich Plasma Stimulates Collagen Type I Synthesis in the Human Skin: A Placebo-controlled *in vivo* Study

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Received: September 23, 2021
Accepted: October 25, 2023

**ABSTRACT** Platelet-rich plasma (PRP) is used in medicine as a source of autologous growth factors in different indications. At present, PRP is applied increasingly frequently in aesthetic medicine with the aim of skin revitalization. Until now, the mechanisms of PRP effects in healthy human skin treated with aesthetic goals have not been identified in detail. This study aimed to examine PRP effects on the synthesis of procollagen type I in human skin. This study was a prospective, single-center, single-dose, open-label, non-randomized controlled clinical study. The study was conducted on a group of 10 volunteers in whom forearm skin was injected with PRP, while the placebo control group received injections of 0.9% NaCl. Expression of procollagen type I was examined after 21 days using immunohistochemistry. The study demonstrated that skin fragments subjected therapy using PRP demonstrated a significantly higher expression of procollagen than that which was observed in placebo controls. The study demonstrated that PRP stimulated collagen expression in healthy human skin.

**KEY WORDS:** platelet-rich plasma, skin, collagen, immunohistochemistry, aesthetic medicine

## INTRODUCTION

Platelet-rich plasma (PRP) represents a concentrate of autologous platelets of a patient, containing around 1 mln. thrombocytes in 1 $mm^3$, an admixture of other blood cells, and proteins, fibrin, fibronectin, and vitronectin. Activated platelets are release by degranulation of numerous growth factors, which are used to initiate wound healing. Until now, around 30 various types have been isolated and estimated, contained in granules of the α of thrombocytes. The most clearly identified among them include PDGF, TGF-β, VEGF, EGF, KGF, IGF, etc. (1-4).

Clinically, PRP has been applied for around 20 years. At the beginning, PRP was applied in maxillofacial surgery, in which it clearly accelerated healing of post-procedural wounds and provided a very good method for filling tissue defects. Good results were also obtained in orthodontics in the treatment of periodontitis. In orthopedics, PRP is used in the treatment of tendon traumas, particularly to the Achilles tendon but also for other tendons. Promising data point to better healing of diabetic foot and other chronic, difficult to heal wounds following

application of PRP. Clinical application of PRP is linked to its favorable effect on wound healing, particularly for chronic wounds, reduced incidence of infections and post-operative pain, and reduced blood loss following procedures of various type (5-12). In plastic surgery, application of growth factors from autologous blood initially focused mainly on application of fibrin-based tissue glues, employed to improve hemostasis, to ensure better attachment of grafted tissue flaps, and as a factor improving fat grafting outcomes (13-16).

In recent years, the application PRP has been introduced to aesthetic medicine on order to revitalize healthy skin. The widespread clinical use of PRP in aesthetic medicine is not supported by either biological studies on the effects of PRP on healthy human skin or clinical studies on statistically significant differences between patient groups. In experimental conditions, PRP was found to reduce formation of wrinkles under effect of UVB radiation (17). PRP has also been show to increase expression of TGF-β protein in the skin of patients with melasma (18). *In vitro* studies have shown that PRP can stimulate fibroblast proliferation and migration (19), collagen and β1-integrin receptors (20), that it induces increased expression of G1 cell cycle regulators, type I collagen, and matrix metalloproteinase-1 in human skin fibroblasts (21), and that it promotes fibroblasts viability, migration, spreading, proliferation, and mRNA levels of known mediators of dermal biology, including PDGF, TGF-beta, and fibronectin (22). Based on histological evidence, PRP injected in the deep dermis and immediate subdermis induces soft-tissue augmentation, activation of fibroblasts, and new collagen deposition, as well as new blood vessels and adipose tissue formation (23,24). Few clinical studies have been conducted on the effects of PRP on healthy human skin. In 2010 PRP was found to significantly improve skin traits subjectively evaluated by patients and measured using several scales (25). Other studies, performed on 31 (26), 11 (27), 27 (28), and 20 (29) patients have shown clinical improvement after different PRP procedures in aesthetic indications.

There are many different PRP harvesting kits available on the market today. They have been classified into leukocyte-poor, leukocyte and fibrin-poor PRP, leukocyte-poor PRF, and leukocyte- and fibrin-rich PRF (30). In addition, the kits differ in anticoagulants, percent of platelet recovery, platelet viability, and different concentrations of growth factors. Presence of growth factors and cytokines and their clinical activity depends on the concentration of the proteins in platelets, the technique of PRP preparation, and on the methods by which activity of the factors is measured (31,32). It has been demonstrated that these parameters can significantly influence their biological activity. It seems to be necessary to determine the precise effects of the individual types of kits (33).

Type I collagen plays a crucial role in the skin. It provides structural support, helps maintain skin firmness, and contributes to its elasticity. This type of collagen is responsible for the overall strength and resilience of the dermis, making it a key component in preventing wrinkles and sagging. Given that the aim of this study was to study the induction of collagen expression, our study assessed the expression of type I procollagen. The aim of this study was to investigate the effect of PRP from one of the popular commercially available kits on the synthesis of type I procollagen in healthy human skin.

## PATIENTS AND METHODS

### Patients

This study was a prospective, single-center, single-dose, open-label, non-randomized controlled clinical study. The study was performed on a group of 10 volunteers (Table 1). The patients provided informed consent before their inclusion into the study. The study protocol conformed to the ethical guidelines of the Declaration of Helsinki as reflected in approval by the institution's human research review committee. PRP and placebo (0.9% NaCl) were placed in a 1 mL syringe (Luer Lock, BD®), coupled to the 30 G needle. The application was performed in a subdermal plane in an area of 1 cm. 1 mL of autologous PRP was injected intradermally into the skin of the left forearm, while 1 mL of placebo was injected into the skin of the right forearm.

**Table 1.** Characteristics of volunteers

| No | Sex | Age | Menopausal status | Signs of photoaging |
|---|---|---|---|---|
| 1 | Male | 39 | - | None |
| 2 | Female | 45 | premenopausal | None |
| 3 | Female | 49 | postmenopausal | None |
| 4 | Female | 33 | premenopausal | Yes |
| 5 | Male | 34 | - | None |
| 6 | Female | 43 | premenopausal | None |
| 7 | Female | 38 | premenopausal | None |
| 8 | Female | 44 | premenopausal | None |
| 9 | Male | 29 | - | None |
| 10 | Female | 31 | premenopausal | None |

## PRP preparation and application

All the PRP was obtained using the commercially available Regeneris Kit (Lea Futur, Poland). Blood from the basilic vein was sampled using a vacutainer. The kit was equipped with a butterfly 21 G needle, a vacutainer kit, calcium chloride, a 2 mL syringe and a 30 G needle. An 8 mL blood sample was aspirated from a patient's peripheral vein in tubes containing sodium citrate anticoagulant. The special 8 mL test tube was prepared. The tubes were equipped with a separator that centrifugally separated red and white cells from PRP. The test tube was centrifuged at 3,000 rpm over 5 minutes. The separating gel yielded around 4 mL of plasma supernatant and a cellular sediment. Using a 1 mL syringe, 0.9 ml PRP was sampled from the tube and mixed in the same syringe with 0.1 mL of 10% calcium gluconate. The obtained suspension was carefully mixed, and the entire content of the syringe was intradermally injected into the skin of the forearm, previously anaesthetized with EMLA cream. The skin of the other forearm, also anaesthetized, was injected with sterile 0.9% NaCl (placebo control) using an analogous needle and syringe.

## Skin biopsies

Twenty-one days later, punch biopsies of the injected sites were performed. Two biopsies were obtained under local anesthesia from each patient. The biopsies were obtained using disposable sterile biopsy punches of a standard type (Miltex Inc, CITO Innovative Solutions, Poland). Subsequently, the material was fixed in 10% buffered formalin and embedded in paraffin.

## Immunohistochemistry

Formalin-fixed, paraffin embedded tissue was freshly cut (4 µm). The sections were mounted on Superfrost slides (Menzel Gläser, Germany), dewaxed with xylene, and gradually rehydrated. Activity of endogenous peroxidase was blocked by 30 min incubation in 1% $H_2O_2$. The sections were boiled for 15 min in a microwave oven at 250W in Antigen Retrieval Solution (DakoCytomation, Denmark). This was followed by immunohistochemical reactions using the monoclonal mouse antibody against procollagen type I (clone Pc8-7; LifeSpan BioSciences, Seattle, WA, USA). The antibodies were diluted 1:100 in the Antibody Diluent, Background Reducing (DakoCytomation, Denmark). Tested sections were incubated with antibodies for 1 h at room temperature. Subsequent incubations involved biotinylated antibodies (15 min, room temperature) and streptavidin-biotinylated peroxidase complex (15 min, room temperature) (LSAB+, HRP, DakoCytomation, Denmark). DAB liquid+ (DakoCytomation, Denmark) was used as a chromogen (7 min, at room temperature). All the sections were counterstained with Meyer's hematoxylin. In every case, control reactions were included, in which a specific antibody was substituted with the Primary Mouse Negative Control (DakoCytomation, Denmark).

**Table 2.** Evaluation criteria of procollagen type I expression using IRS (ImmunoReactive Score)

| Percentage of positive cells | Points | Intensity of reaction | Points |
|---|---|---|---|
| No positive cells | 0 | No reaction | 0 |
| <10% positive cells | 1 | Weak reaction | 1 |
| 10-50% positive cells | 2 | Moderate reaction | 2 |
| 51-80% positive cells | 3 | Intense reaction | 3 |
| >80% positive cells | 4 | | |

## Scoring of immunostaining results

The intensity of the immunohistochemical reactions was appraised using the semi-quantitative immunoreactive score (34), in which the intensity of the reaction and the percentage of positive cells were considered. The final result represented a product of scores given for individual traits and ranged from 0 to 12. The intensity of the reactions was evaluated independently by two pathologists. In case of divergences, the evaluation was repeated using a double-headed microscope (Table 2).

## Statistics

Statistical analysis of the results was performed using Statistica 2007 PL (Statsoft, Kraków, Poland) software. In order to compare the intensity of procollagen expression on PRP-injected skin and in the

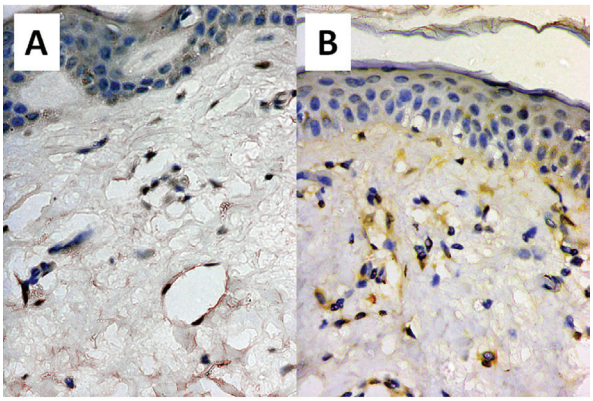
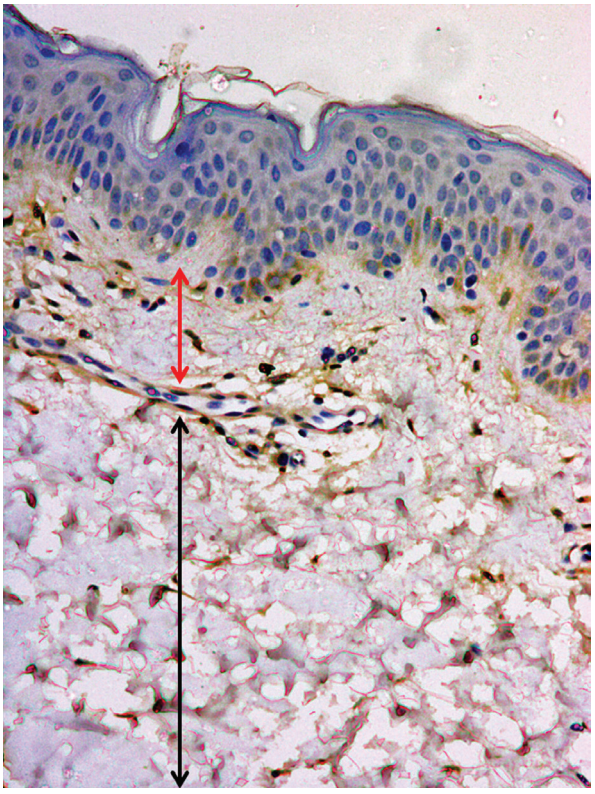



**Figure 1.** Immunohistochemical localization of procollagen type I expression (brown color) in: (A) placebo-treated skin; (B) PRP-treated skin of the same patient.



placebo control and to compare the intensity between individual clinical subgroups treated with PRP, Mann-Whitney U-tests of and Kruskal-Wallis ANOVA rank tests were employed. *P* values <0.05 were considered to indicate significant differences.

## RESULTS

The conducted immunohistochemical reactions resulted in a color reaction located in the cytoplasm of fibroblasts. The intensity of the reaction varied between the preparations. In the PRP-treated group, mean intensity of the reaction was to 9.28±1.84 SD in the IRS score, while in the placebo group it was 2.02±2.54 SD (IRS score) (Figure 1).

The intensity of procollagen type I expression proved to be higher in the papillary dermis compared with the reticular dermis (Figure 2).

Statistical analysis demonstrated that expression of procollagen type I was significantly more pronounced in the skin samples subjected to autologous PRP compared with the placebo-treated skin (*P*=0.0012) (Figure 3).

Analysis of the relationships between the intensity of the studied protein expression on one hand and age or sex on the other demonstrated no significant differences. However, due to the very small sample of examined individuals, the result cannot be regarded as reliable.

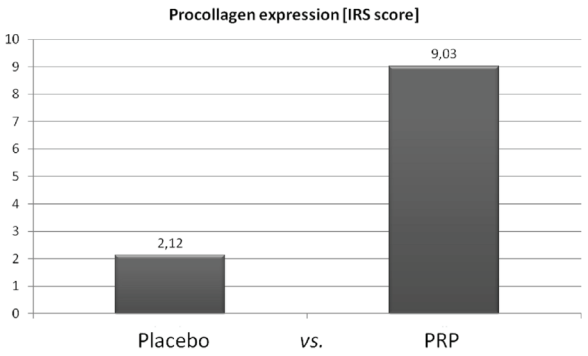



**Figure 3.** Mean expression of procollagen type I in PRP-treated skin and in placebo-treated skin.

No unfavorable effects were observed in any of the examined cases. The patients only complained of a slight discomfort at the site of administration, persisting for a few hours. Additionally, reddening and a slight edema on the injection site lasting for a few hours after administration were noted.

## DISCUSSION

In aesthetic medicine, PRP is currently used to stimulate regenerative processes in the skin and to achieve a revitalization effect. Few experimental studies point to a potential for skin-targeted effects of PRP. Krasna *et al*. found that PRP stimulates proliferation of fibroblasts *in vitro* (35). Other studies demonstrated *in vitro* that PRP stimulates collagen expression in cultured cells of anterior cruciate ligament (36,37). Kim *et al*. (38) used cultured human dermal fibroblasts to show that PRP induces cell proliferation. Additionally, PRP increased the expression of type I collagen, MMP-1 protein, and mRNA in human dermal fibroblasts. Cho *et al*. also performed a cell culture experiment (21) showing that PRP treatment induced increased rates of cell proliferation and cell migration. In addition, expression of Rb, cyclin E, and cyclin-dependent kinase 4 proteins was increased by a high concentration (5%) of PRP-treated human skin fibroblasts. High concentration of PRP induced an up-regulation of type I collagen, MMP-1, and MMP-2 expression in studied fibroblasts.

In treatments aimed at improving the quality of the skin, PRP is used either as monotherapy or in combination therapy. In the case of monotherapy, PRP is administered in the form of mesotherapy, i.e. numerous microinjections into the treated area. An interesting observation arose in the work of Redaelli *et al*. (20). This study on the effectiveness of PRP monotherapy carried out on a group of 23 patients

yielded interesting results. Patient satisfaction with the procedure performed was higher than that of the physician. These data show that patient satisfaction is important in assessing the effectiveness of this type of therapy, because this parameter is related to the subjective feelings of the patient that are not measurable for the physician. Significantly more data exist when PRP is used in combination with other methods, mainly with procedures based on the production of microtraumas. In this type of surgery, PRP was usually used to treat atrophic scars and stretch marks. Significantly higher effectiveness of treatments combined with PRP compared with monotherapy with the use of microneedling or various fractional lasers was described (39-43).

As mentioned above, due to the large differences between individual PRP kits, each should be tested separately. In the case of studied kit, the study by Abuaf *et al*. (24) showed that after 28 days from the injection, the skin of patients had a greater amount of collagen fibers compared with the placebo group. Collagen was detected by Masson's thichrome histological staining.

We studied the procollagen expression 3 weeks after injection because procollagen type I appears in the early stages of the wound healing process, typically within the first few days following injury or surgery. It is a form of collagen produced in response to skin or tissue damage. Pro-collagen is later transformed into mature collagen, which is crucial for rebuilding tissue structure and restoring full strength. The wound healing process is complex and involves multiple stages, with collagen type I playing a key role in the formation of new collagenous tissue and the restoration of skin integrity. Already in the early phase of inflammation, fibroblasts begin to synthesize new collagen under the influence of TGF and PDGF. It is believed that the process of synthesis of this component of the extracellular matrix begins 4 to 5 days after injury. In the 3rd week, the process of synthesis is intensified in most cases. Taking into account the fact that we determined the expression of procollagen in our study, the time of biopsy seems to be adequate. In our work, based on immunocytochemistry performed 21 days after injection, we showed significantly higher expression of type I procollagen in the skin of patients treated with PRP compared with placebo. The phenomenon has been documented in all patients. Due to the limited size of the group, no relationships could be documented between age, sex, and menopausal status on one hand and sensitivity to PRP on the other. No serious undesirable effects of PRP were detected in the present study. Determination of procollagen is better evidence of the stimulation of collagen expression in comparison with classical histological tests, especially in such small groups of patients. Expression of procollagen type I in PRP-induced skin has been found to be more pronounced in papillary dermis as compared with reticular dermis. The phenomenon most probably reflectes the fact that papillary dermis contains less differentiated cells, with potentially higher sensitivity to growth factors (44).

## CONCLUSION

In summary, we have demonstrated that PRP obtained from the studied kit stimulated expression of procollagen type I in the human skin. The study indicated that PRP injections may represent an interesting skin-revitalizing procedure. For an appropriate clinical application, examination of a few additional aspects the method is required:

- the relationships between the technique of the procedure, volume of injected PRP, and the efficacy of the procedure
- the relationship between patient age and the efficacy of PRP
- determination of PRP dosage: how frequently and how many times the procedure should be applied in a series to obtain the desired effects
- in the next study, biopsies should be taken in the treated area 3 months and not 3 weeks after the treatment, since most of the new collagen needs a fairly long time to develop. We cannot be sure if the symptoms of the start of new collagen production result in a clinical improvement. If such a study is performed, visualization using OCT, ultrasound, or confocal microscopy could also help evaluate the collagen increase in a non-invasive way.

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