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# THE USE OF PLATELET-RICH PLASMA IN ANTI-AGING THERAPY

# /overview/

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## ABSTRACT

### Introduction

Aging stands for the changes in the human body, which are recognized as natural processes that concern every person and are related to the passage of time. Despite ‘normalness’ of the process, a tendency is emerging to treat aging as a pathology requiring proper diagnostics. Consequently, an ‘anti-aging’ medicine has been established - defined as study of preventing and slowing down the process of aging.

### Aim of the study

The aim of this study is to summarize and present the current knowledge, based on the overview of writing on the properties and effectiveness of the use of PRP in anti-aging therapy.

### Description of knowledge

Autologous platelet-rich plasma is a concentrate of human blood platelets suspended in a small amount of plasma, wherein concentration of thrombocytes is higher than initial concentration. For autologous plasma to have high healing properties, platelet levels should be at least 1 million in 5 ml of plasma.

The basic condition for initiating repair and regenerative processes in vivo is the formation of a blood clot. Also, by degranulating, platelets release many growth factors, such as PDGF, TGF-β, EGF, FGF, VEGF and others, which are involved not only in blood coagulation, but also in immune response and tissue healing.

The large variety of PRP-obtaining methods and the range of concentration levels of growth factors in PRP may lead to ambiguity in the literature, as to the extent to which formulation was actually used in a variety of preclinical and clinical studies. Some of those studies are presented in this article.

### Summary

Platelet-rich treatments are part of the growing popularity of regenerative medicine, which is beginning to be recognized as the medicine of the future. However, without proper control methods or optimization of numerous variables (e.g. concentration or ratio of growth factors) during PRP preparation, it is not known which ingredients are needed and preferred, and which are harmful for tissue regeneration.
However, numerous studies show PRP injection as a promising method of rejuvenating the skin, therefore platelet-rich treatments are an important part of the growing popularity of regenerative medicine.


## INTRODUCTION AND THE AIM OF THE WORK:

Aging stands for the changes in the human body, which are recognized as natural processes that concern every person and are related to the passage of time. Despite ‘normalness’ of the process, a tendency is emerging to treat aging as a pathology requiring proper diagnostics. Consequently, an ‘anti-aging’ medicine has been established - defined as study of preventing and slowing down the

process of aging. Its goals are realized by creating optimal health conditions and choosing the right lifestyle and supplementation.

Because of the need to be beautiful and attractive, it is understandable that many "anti-aging" efforts focus on maintaining a healthy appearance and skin condition. This action is all the more important because patients largely do not accept the physical changes that occur during aging and often cannot accept the change in their appearance.

The therapy itself is hindered by multitude of factors connected with aging. Among them we distinguish factors both exogenous (e.g. aging of the skin as effect of ultraviolet radiation) and endogenous (connected with genetic and hormonal agents).

'Anti-aging' medicine worked out a variety of treatments which aim is to stop, or even undone the aging of the skin. One of them is a treatment using platelet-rich plasma (PRP), which has recently become hugely popular in a number of fields of medicine. First attempts of PRP treatment indicate that it can be an effective curative.

The aim of this work is to summarize and present the current knowledge, based on the overview of papers on the properties and effectiveness of the use of PRP in anti-aging therapy.

## DESCRIPTION OF KNOWLEDGE:

### What is platelet-rich plasma?

Autologous platelet-rich plasma is a concentrate of human blood platelets suspended in a small amount of plasma [1], wherein concentration of thrombocytes is higher than initial concentration [2]. Other names of PRP are: growth factor-rich plasma or autologous concentrated plasma.

The first PRP formulations were developed in the 1970s, while the first efficient method of preparation was developed by Whitman et al. In 1997 [3].

Blood platelet levels should be 150-400 thousand per microliter. As Marx suggested, plasma can only be called platelet-rich if the concentration of concentrated platelets exceeds the baseline. For autologous plasma to have high healing properties, platelet levels should be at least 1 million in 5 ml of plasma. The use of formulations with lesser amount of thrombocytes does not significantly affect the healing process [2].

### Platelet-rich plasma's biology

The basic condition for initiating repair and regenerative processes in vivo is the formation of a blood clot. This property is used to administer PRPs with significantly higher thrombocytes concentrations, which facilitates clot formation and speeds up the treatment [3]. A comparison of blood composition and PRP is shown in Table 1.

| Component | Whole blood | PRP |
|---|---|---|
| Red blood cells | 95% | 4% |
| White blood cells | 1% | 1% |
| Platelets | 5% | 95% |

**Table 1. Comparison of whole blood composition and PRP, based on Gurpreet et al.**

## Platelet's biology

Thrombocytes are the smallest, non-nuclear blood cells, which are produced as fragments of the megakaryocytes cytoplasm in the thrombopoiesis. The thrombopoietin is the most important regulating factor in the process.

The most important role of platelets is their involvement in hemostasis by adhesion and aggregation on the wall of the damaged vessel, and the formation of a primary platelet plug. In addition, they take part in: synthesis, storage and release of many different compounds (e.g. thrombin, adenine nucleotides, serotonin, $Ca^{2+}$ ions), vascular wall remediation, wound healing, triggering of inflammatory, immune, atherosclerotic and neoplastic reactions [4]. At the same time, they are also a reservoir of many growth factors involved in blood coagulation, immune response and tissue healing [7].

The most important growth factors stored in thrombocytes are:
**1. PDGF (Platelet Derived Growth Factor)** – The first described growth factor involved in healing of wounds.
PDGF – occurring in 5 isoforms- is considered to be one of the most important and most potent wound healing stimulators. Its assistance occurs already in the first hour and lasts until a wound is healed. Its main source is de-granulating blood platelets, but it can also be produced by fibroblasts, keratinocytes, endothelial cells, and macrophages. It has a chemotactic effect on the stem cells, present in the bone marrow, as well as on neutrophils, monocytes and fibroblasts. The latter are activated by PDGF for proliferation and for the production of extracellular matrix components. The studies relating to the direct application of PDGF for wound showed that they cause more rapid healing and appearance of granulation tissue, and also increase the mechanical strength of the skin [5]. Simultaneously, in patients with delayed healing and diabetic foot, a reduction of concentration of PDGF has been observed [6]. Defects of high concentrations of PDGF are, however, the tendency for keloids to form and its significant contribution to neoplastic processes [8].
2. **TGF-β (Transforming Growth Factor β) -** produced mainly by thrombocytes, just after tissue injury. It exhibits chemotactic effects on macrophages, fibroblasts and neutrophils. It is also a potent myogen for skin fibroblasts and stimulates the migration of keratinocytes. This cytokine also participates in the stimulation of the synthesis and degradation of extracellular matrix proteins and regulates the entry of cells into the apoptosis pathway. [6,5]
**3. EGF (Epidermal Growth Factor) -** Its action is to stimulate the migration and proliferation of epidermal cells. It accelerates skin metabolism and promotes the transport of active nutrients, resulting in increased collagen synthesis. It is also responsible for repairing damaged polysaccharides and glycoproteins that build cell membranes [8].
**4. FGF (Fibroblast Growth Factor) -** active mitogen, which stimulates the proliferation of many cells. Basic Fibroblast Growth Factor (FGF-2) is an especially important factor - it is produced by damaged endothelial cells of the blood vessels immediately after injury or severe burns. It stimulates mature endothelial cells and their precursors to migration and mitotic division [9].

**5. VEGF (Vascular Endothelial Growth Factor) -** one of the most active angiogenesis and vasculogenesis inducers, in addition to thrombocytes produced in keratinocytes, macrophages, fibroblasts and neutrophils. This cytokine acts via receptors mainly on the surface of endothelial cells, triggering the signal that stimulates angiogenesis. This results in the construction of new vessels on the scaffold composed of collagen and other proteins of the extracellular matrix. VEGF was also shown to participate in the production of collagen fibers and stimulation of thrombocyte agglomeration and clot formation [9].
**6. IGF (Insulin-like Growth Factor) -** its activity mainly involves the activation of keratinocytes and the stimulation of epidermal cell proliferation
**7. HGF (Hepatocyte Growth Factor) -** responsible for regulating migration and differentiating stem cells of the epidermis [9].
Due to the particularly high content of these factors in the formulations of platelet-rich plasma, they have been used in various fields of medicine, including the treatment of chronic, difficult to heal wounds, soft tissue injuries, maxillofacial surgery, implantology, periodontics, burns, plastic surgery and aesthetic dermatology as one of the main anti-aging therapies [10].

**The aging of the skin**

Aging of the skin has characteristic features. There is, among other things, atrophy of the epidermis and skin - the dermis thickness decreases with age by about 20%, and the thickness of the stratum corneum does not change with age. As a result of aging, the number of fibroblasts and mast cells decreases. In "young" skin collagen is arranged in the pattern of a regular grid, while in aging skin collagen is arranged in tufts giving the impression of disorder. Elastic fibers, in turn, undergo calcification and fragmentation in the extracellular matrix, the amount of the metalloproteinase enzyme is increased, leading to its degradation, as well as the accumulation of material containing malformed elastin in the papillary and reticular dermis.

Over the years, dermal-epidermal junction (DEJ) undergoes many changes: the amount of collagen IV and VII decreases, leading to the dilution of this junction and, consequently, the formation of wrinkles. Turn-over time is prolonged, healing becomes slower and skin elimination becomes less effective. As a result of aging, the amount of hyaluronic acid in the skin is almost completely reduced. Hyaluronic acid has water-binding properties, which allows the skin to maintain a water-electrolyte imbalance.

In "young" skin hyaluronic acid is found on the periphery of collagen and elastin, while in the skin undergoing aging, such connections disappear. The decrease of collagen, elastin and hyaluronic acid levels is responsible for formation of wrinkles and furrows, as well as for dryness and looseness of the skin [11].

The aim of the standard methods of preventing skin aging is to increase the production of extracellular matrix by activating fibroblasts. Platelet concentrate is a rich source of a wide range of cytokines and growth factors, which activation occurs after injection into a selected tissue fragment. Platelets are activated endogenously by coagulation factors (in some methods of preparing, PRP is activated just prior to administration to the tissue).

Then, by combining agents with special cell surface receptors, certain intracellular processes are activated, which allows the production of extracellular matrix, and also improves cell proliferation and differentiation. Tissue regeneration is the result of regeneration, migration and proliferation of relevant cells [12].

**MMPs - Matrix metalloproteinases -** form part of all tissues and organs in the human body. The expression and production of metalloproteinases is found in almost all cells: fibroblasts, keratinocytes, macrophages, endothelial cells, Langerhans cells, neurons, myocytes, monocytes, leukocytes, neutrophils and tumor cells. Metalloproteinases play a very important role in both physiological and pathological processes. They take part in, for example, the healing of wounds and the formation of scars. They also participate in the processes of angiogenesis and apoptosis. Metalloproteinases are also involved in pathological processes: cancer, autoimmune diseases. They participate in the etiology of a number of skin diseases. Recent reports include the presence of metalloproteinases in the etiopathogenesis of skin aging [13].

The main area of activity of metalloproteinases is the hydrolysis of the components of the extracellular matrix (ECM), primarily collagen, and the removal of ECM in the process of resorption and tissue reconstruction. Studies conducted on cell culture and human skin model have shown that the primary source of MMPs that respond to UV radiation are fibroblasts derived from dermis. It is possible that fibroblasts may play a significant role in the production of MMPs, by indirect mechanisms involving the release of growth factors and cytokines, which affect the production of MMPs in keratinocytes [14]. One of main factors leading to the stimulation of metalloproteinase activity in aging process is UV radiation. The effects of metalloproteinases are changes in the structure of the dermal matrix, wrinkles, decrease in elasticity and skin firmness [15].

The other study showed, that high PRP concentration increases levels of type I collagen and enhances the expression of metaloproteinases in human fibroblasts [12]. Mechanisms, described above indicato connection between PRP and proliferating mesenchymal stem cells [10].Another study has shown that high PRP levels increase the amount of type I collagen, and increase expression of metalloproteinases in human fibroblasts [12]. The above mechanisms for improving the condition of aging skin using PRP show the interactions between platelet concentrate and proliferating mesenchymal stem cells [10].

Another mechanism of positive effect of PRP on skin rejuvenation is the accelerated synthesis of hyaluronic acid. This acid absorbs water, which results in an increase in the volume of the cell matrix, which in turn leads to increased volume and tension of the skin, as well as promotes cell proliferation, ECM production and helps to adjust the cross-section of collagen fibers. All these elements affect the elasticity of the skin [10].

| **Contraindications for platelet-rich plasma treatment:** | |
|---|---|
| 1. | Pregnancy and breast-feeding. |
| 2. | Under 18 years of age or over 65 years. |
| 3. | Smoking of tobacco products. |
| 4. | Immunosuppression. |
| 5. | Emotional instability, mental illnesses |
| 6. | Disorders in the coagulation system. |
| 7. | Number of platelets below 100 000 / ul. |
| 8. | Purpura |
| 9. | Some infectious diseases. |
| 10. | Use of anticoagulants. |
| 11. | Use of NSAIDs within 10 days before the planned treatment. |
| 12. | Use of corticosteroids. |
| 13. | Susceptibility to keloids. |
| 14. | Hemoglobin levels below 12 g / dl in women and 14 g / dl in men. |
| 15. | Active inflammation at the site of the planned surgery. |
| 16. | Some chronic diseases: chronic renal failure, diabetes, hepatic insufficiency / hepatitis, cardiovascular failure. |

**Table 2 Contraindications for platelet-rich plasma treatment:**

**Methods of obtaining platelet-rich plasma.**

There are a number of commercial systems that help to prepare platelet-rich suspension in a recurrent manner. All of them work on the basis of drawing a small amount of venous blood (about 20 to 60 ml), most often from the upper limb vein. The patient does not have to be on the empty stomach. Blood extracted in a sterile manner is collected in a special separator (container used to obtain PRP). The collected material is then centrifuged in a special laboratory centrifuge

The plasma separators are different. Some are simple centrifuges, some high-end, complex, dual-centric systems [17].

There are two methods of obtaining a platelet gel:

1. double spin method.
2. buffy coat method[18].

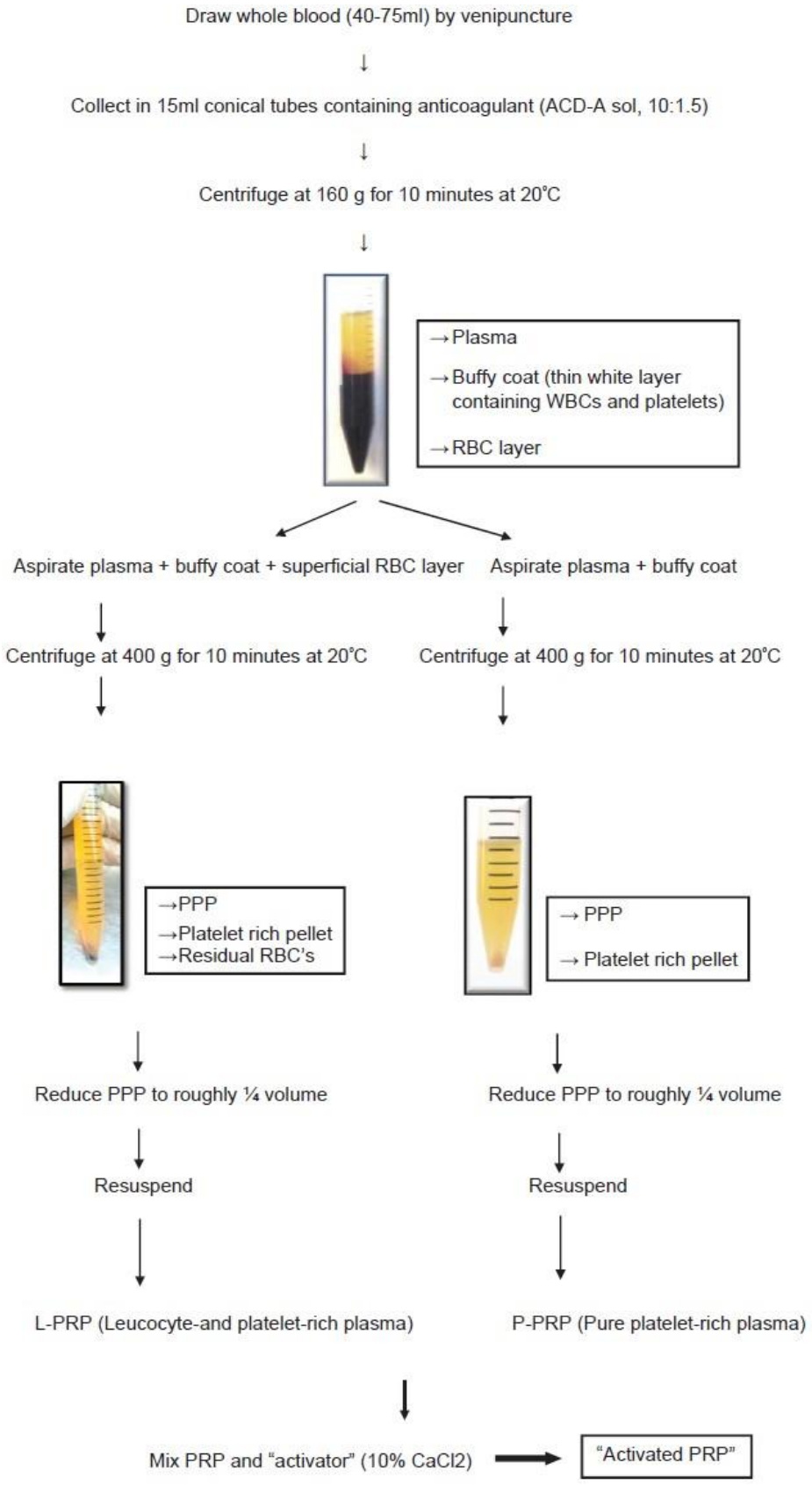


Fig. 1 Methods of obtainging PRP [18]

**Clinical aspects of PRP usage in anti-aging therapy.**

Plate rich plasma has long been known for its effectiveness in many areas of medicine. The purpose of this study was purely practical. It was assessed whether there were real effects, benefits and side effects of PRP injection in the face and neck area.

23 patients (mean age 47 years, range 28-70) participated in this study lasting 3 months. Injections with platelet rich plasma was performed once a month. Regen Lab was used for the preparation of concentrated platelets.
The parameters that have been evaluated were nasolabial folds, skin elasticity assessment, skin homogeneity and texture, wrinkles around the eyes, wrinkles on the neck2. The results were evaluated one month after the last session. Each patient received 4 ml of calcium chloride- activated PRP. 0.1 ml of calcium chloride was used on every 0.9 ml of PRP. Platelet concentrate was injected within 7 minutes.

| | |
|---|---|
| Nasolabial folds | 1ml / 0,5 ml per side |
| Neck bands | 1ml / 0,5 ml per side |
| Periocular wrinkles | 1ml / 0,5 ml per side |
| Cheeks | 1ml / 0,5 ml per side |
| Forehead | 1ml / 0,5 ml per side |

**Fig 3. Places and volumes of PRP injection in presented study.**

In the study cited no serious and long-lasting side effects were observed. Among mild and transient adverse event that were observed were: acceptable bruises and petechiae(3% of patients), a burning sensation lasting for about 3 minutes after injections (70% of patients) and mild erythema in the place of injection(80% of patients).

The results of the study were evaluated one month after the last session, taking into account: pre and postoperative images (using a dermoscope and a digital camera), satisfaction of the patient and physician.

Recived results:

| % of Patients: | Result: | Score: |
|---|---|---|
| 0,00% | None | 0 - 4 |
| 17,00% | Mild | 4 - 8 |
| 70,00% | Good | 9 - 16 |
| 13,00% | Very good | 17 - 24 |
| 0,00% | Excellent | 25 - 36 |

**Table 4. Results obtained in the form of a table.**

| | |
|---|---|
| Nasolabial folds | 24% |
| Neck bands | 28% |
| Skin homogeneity and texture | 33% |
| Skin tonicity | 22,5% |
| Periocular wrinkles | 30% |

**Table 5. Improvement in% of individual parameters tested according to individual parameters.**

Each patient in the study experienced significant improvement. The average score was between good and very good.

To summarize the study, it should be noted that facial and neck revitalization with platelet concentrate is a simple and promising procedure performed with positive results in all fine wrinkles of the skin, improving its structure and elasticity. Very good results were also observed in skin homogeneity. The technique of injection is well tolerated by patients. No permanent or severe side effects have been observed. Satisfaction of the patients was significant. The exact mechanism of action of the injection technique has not yet been fully elucidated. Although PRP injection methods require further verification, initial results are very promising and encouraging, especially in cases where corrective procedures are required [16].

Another study of PRP usage which we would like to cite [in this article] concerns the effect of PRP on the periorbital dark circles. This defect affects people in different ages, both sexes and all races. It also intensifies with the progression of aging. This study was the first to measure the non-invasive and objective effect of glaucoma as a rejuvenating agent for periorbital dark circles and for reducing "crow's feet". This study involved 10 patients with a mean age of 41.2 years, with skin type III and IV according to the Fitzpatrick classification. Injections were done intradermally; 1.5 ml of PRP was injected to the infraorbital area and within the "crow's feet". Control visits and reevaluations were scheduled for 7 days, 1 month and 3 months after surgery.

The results of the study were unequivocal. The improvement of homogeneity of the color of the subcellular area was statistically significant, but there were no significant changes in melanin content, hydration of the epidermis and depth of wrinkles. 80% of patients achieved a good improvement after 3 months. The average score in the applied scale was 1.7 (range 0 - 3). 90% of the patients rated their results as excellent or good. The average satisfaction score was 2.2 (range 0-3) three months after surgery.

In addition to mild itching observed in all participants, 60% of patients had benign and transient petechiae, occurring in the first week after injection. None of the patients reported a lasting, significantly adverse side effect.

Analysis of the results of the study shows that platelet concentrate appears to have a great potential for improving periorbital dark circles in the context of unification of skin color. Intradermal injection with PRP shows promising effects on the periorbital dark circles, but in terms of wrinkles around the eyes, the results are not very promising.

The last, and also the biggest study that will be described in this publication is the use of PRP in the broadly understood "facial rejuvenation". This study was conducted by 3 doctors from different countries. My Cells Autologous Platelet Preparation Kit, which is often used in corrective and

aesthetic dermatology in the face area, was used to obtain a platelet-rich formulation. MyCells Kit was authorized for use in 2009 by the FDA and approved by the European Union and the Israeli Ministry of Health. The results of a pilot study of 10 women showed that platelet-rich plasma injection for facial rejuvenation is effective and safe, especially in areas of the face considered "difficult", such as eye contour and neck. Further clinical trials were conducted with MyCells Kit in Japan, the United Kingdom and Israel, where PRP was given to 408 patients (including 387 women and 21 men). The clinical effects of facial rejuvenation and potential side effects were evaluated.

| COUNTRY: | INVESTIGATOR: | AGE RANGE OF PATIENTS (years): | NUMBER OF PATIENTS: | NUMBER OF WOMEN/MEN: |
|---|---|---|---|---|
| Japan | Junichiro Kubota (Tokyo) | 38-72 | 172 | 159/13 |
| Great Britain | Jacques Otto (Londyn) | 42-79 | 194 | 186/8 |
| Israel | Amos Leviav (Tel Aviv) | 46-74 | 42 | 42/0 |

**Tabel 6. Summary**

In trials, PRP has been used to stimulate the superficial layer of dermis, as well as deeper layers. To stimulate the superficial layer of skin, the injections were made shallow, in the form of classic mesotherapy to lighten, hydrate and improve the skin quality. In these cases, a 32-gauge needle was used. For stimulation of deeper skin layers, PRP injections were performed according to the technique used for the use of fillers, using a 30-gauge needle. The amount of PRP used was between 4 ml and 6 ml depending on the patient. After injection, Auriderm XO - Auriga - a blood vessel sealing cream or equivalent was used.

Patients were evaluated at 3 month intervals. The results were dependent on the age of participants. In younger patients (less than 35 years), there was a faster response to treatment. Their main indication was rejuvenation and prevention of skin aging. For this age group, therapy every 12 to 24 months has proven to be sufficient. Patients up to 45 years of age required a second treatment 9 to 12 months after the first treatment plus annual injections combined with another treatment. Patients aged 50 to 60 required a second treatment after 6 months, a third treatment after 15 months after the first treatment, and 2 years after the first surgery required a remission. A group of patients aged over 60 years needed a second treatment after 3 months, a third treatment after 9 months, and a fourth treatment one and a half years after the first.

Compared with other skin anti-aging therapies, clinical experience with PRP in the cited study has shown that it is useful as a basic or supportive tissue rejuvenation therapy. Both superficial and deep skin application can cause skin rejuvenation and global volume increase. PRP is a biostimulator that is safe and creates an immediate and long lasting volume effect, extracting beauty from the natural look. The technique of injection is easy to implement and practically devoid of side effects. Injections with PRP guarantee high patient satisfaction [18].

## SUMMARY AND CONCLUSIONS:

The use of platelet-rich plasma in anti-aging therapy is now a subject of many studies and discussions. The results of some of the studies are debatable, while others exclude each other. This can be due to several reasons. The large variety of PRP-obtaining methods and the range of concentration levels of growth factors in PRP may lead to ambiguity in the literature, as to the extent to which formulation was actually used in a variety of preclinical and clinical studies. Attempts have been made to classify different platelet concentrates, but different definitions are often used interchangeably, resulting in confusion. In addition, many devices prepare the PRP formulation that exhibits high variability, both in terms of concentration of growth factors and specific cell contents (e.g. leukocytes).

Without proper control methods or optimization of numerous variables (e.g. concentration or ratio of growth factors) during PRP preparation, it is not known which ingredients are needed and preferred, and which are harmful for tissue regeneration. Consequently, the results of each in vivo study refer to one specific method of preparation (application method), which makes the comparison of results of various studies virtually impossible [19]. In many PRP acquisition systems, the clinically significant layer is separated by the operator by means of a needle and a syringe, which makes the actual concentration of growth factors and platelet counts unique and not definable, and the PRP acquisition technique is very sensitive to the operator's senses.

In order to better understand the complexity of the platelet-rich plasma activity and the possible differences in results of subsequent publications, it is important to recall the general mechanism of action of these preparations. Prior to the clinical application of platelet-rich plasma to the treated sites, polymerization of the fibrous tissue is artificially induced by the addition of thrombin (usually bovine) or calcium ions - the resulting fibrin network differs in the structure of fibrin single-stranded connections, which affects the cytokine release and growth factors. Artificially activated PRP releases agents almost immediately and throughout their volume at the time of application of the material [19] - approximately 70% of growth factors are released within the first 10 minutes, and 100% of factors within an hour. The use of the above-mentioned calcium chloride increases the time of release of growth factors from PRP [19]. The use of PRP activated by bovine thrombin, although cheaper, can lead to undesired response of the recipient's immune system. This means that a more secure option is to use autologous thrombin or not to administer non-activated PRP - there is no consensus as to whether the platelets must be activated prior to use. Some activate the platelets by adding thrombin or calcium chloride, while others use PRP without activating, claiming that they get better results. Collagen as a natural PRP activator should also be mentioned. [20]

The potential for skin renewal, which is contained in platelet-rich plasma, cannot be compared to any of the cocktails used during mesotherapy. Growth factors enclosed in the blood platelets, and released during the administration of PRP concentrate into the skin force the patient's body to regenerate and rebuild. The effect that we can achieve by this is primarily the reduction or significant reduction of wrinkles, improvement of skin tension and elasticity, as well as a significant improvement in the color of the skin, thanks to the formation of new blood vessels to nourish the skin. Moreover, what is important, platelet-rich plasma, being a biocompatible treatment that uses the patient's own potential - his blood - reduces the risk of intolerance, which can occur after administering foreign substances to the body, such as those found in cocktails used during mesotherapy.

This is a safe method for everyone, also for people with allergies who are more prone to allergic reactions. Platelet rich plasma therapy is commonly associated with other aesthetic medicine

treatments. A good example is the use of plasma before and after laser invasive surgery as a complement to liposuction or tissue transplantation. The most advanced trends in aesthetic medicine are currently therapies using both regenerative and stem cells. It takes place in the combination of platelet rich plasma with a fat graft. The stem cells contained in the adipose tissue have the same ability as the PRP to regenerate the skin. They can be used to rejuvenate the whole body. Platelet-rich treatments are part of the growing popularity of regenerative medicine, which is beginning to be recognized as the medicine of the future.

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