POSSIBILITIES OF ATROPHIC POST-ACNE SCARS THERAPY WITH THE COMBINED USE OF PRP-THERAPY, T-LAB PRP-TUBE AND THE PRODUCT COLLOST®

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Acne is the most common dermatosis among young, socially active people. Up to 35 % of male adolescents and 23 % of female adolescents suffer from this disease. The long-term course of the disease, especially on exposed areas of the body, leads to psychoemotional stress in most patients: the patient's assessment of the severity of acne is often significantly overestimated compared to the actual clinical course of the disease.

Acne is a multifactorial chronic recurrent inflammatory disease of the sebaceous hair follicle.

The key factors in the development of the pathological process are lipid imbalance, increased pathogenicity of *Propionibacterium* and activity of the sebaceous glands, disruption of the circulation of certain hormones, hereditary predisposition, follicular hyperkeratosis, and disruption of keratinization processes.

Acne disease is one of the most common causes of scar formation, as well as disturbances of natural skin pigmentation, which can be combined into one term “post-acne”. Post-acne includes a symptom complex of secondary rashes that developed as a result of the evolution of various forms of inflammatory acne and are accompanied by pigmentation disorders and the formation of cicatricial changes in the skin.

Long-term severe acne increases the risk of developing post-acne, which can reach 95 %, and with some forms of acne (papulopustular and nodulocystic), post-acne is observed in 100 % of patients [1–3].

The resulting consequences of acne, sometimes disfiguring, bother patients no less than the active manifestations of the disease itself, are difficult to correct, require expensive long-term treatment, can persist for life, and reduce the quality of life of patients [4–5].

Biochemical and pathophysiological processes of post-acne scars formation

According to modern concepts of scar pathogenesis, the evolution of inflammatory elements of acne with the outcome in an atrophic or hypertrophic scar is associated with an imbalance of metalloproteinases responsible for the architecture of the extracellular matrix: MMP-1, MMP-2, MMP-9, MMP-13, proMMP-1, proMMP-9, MMP-28 (epilysin).

Matrix metalloproteinases belong to a large family of Zn^{2+}- and Ca^{2+}-containing endopeptidases secreted by various types of cells that destroy all protein and proteoglycan components of the extracellular matrix, including fibrillar and non-fibrillar collagens, fibronectin, laminin, and glycoproteins of the basement membrane [6, p. 33].

The totality of all MMPs is capable of hydrolyzing all components of connective tissue. The activity of various MMPs has a wide range of biological consequences, since they destroy most components of the extracellular matrix: interstitial collagens and collagens of the basement membrane, proteoglycans, decorin, fibromodulin, fibronectin, etc.

MMP gene expression is induced by a large number of substances, including growth factors, cytokines, chemical agents, and mechanical stress. The sources of MMPs formation in the skin are fibroblasts, macrophages, neutrophils, monocytes, and keratinocytes. In addition to matrix proteins, the substrates of MMP action are growth factors and their receptors (transforming growth factor b, fibroblast growth factor, epidermal growth factor), cytokines (tumor necrosis factor a, interleukins), adhesion molecules (integrins) and apoptosis factors. This apparently explains not only the regulatory function of MMPs in the mechanisms of degradation/accumulation of the extracellular matrix, but also their indirect participation in intercellular and cell-matrix interactions, in morphogenesis, angiogenesis, proliferation, migration and differentiation of cells, and apoptosis [7, pp. 19–22].

MMP-1 collagenase I is synthesized by fibroblasts, macrophages, keratinocytes, endothelial cells, etc. MMP-1 synthesis is stimulated by various agents: epidermal growth factor, interleukins and TNF-a, chemical compounds such as cAMP. MMP-1 is involved in the degradation of collagen strands during extracellular matrix remodeling.

The process is aggravated by the cell wall peptide *P. acnes*, which enhances the degradation of the extracellular matrix through gene expression of proMMP-2 synthesis [8, 9, 10].

MMP-2 (gelatinase) is expressed primarily in fibroblasts during tissue development and regeneration. It is also synthesized by neutrophils, macrophages and monocytes. MMP-2 is required for angiogenesis process inhibition. Together with MMP-9, it is involved in the degradation of type IV collagen, the main component of basement membranes and gelatin.

MMP-2 can also degrade other types of collagen (V, VII, and X), elastin, and fibronectin. For example, MMP-2 cleaves monocyte chemotactic protein-3, which results in decreased inflammation and provides vasoconstriction.

MMP-9 (also known as gelatinase B), along with elastase, is a regulatory factor for neutrophil migration across the basement membrane during inflammation. MMP-9 has several important functions in neutrophil function, such as extracellular matrix degradation and TGF-b activation. Transforming growth factor TGF-b initiates apoptosis in most cell types. TGF-b can induce apoptosis by activating either of two signaling pathways – SMAD or DAXX.

Substrates for MMP-9 include denatured collagen type I (gelatin), native collagens of types IV, V, VII, X, and XI, fibrinogen, entactin, which binds laminin (a key component of the cell membrane), and type IV collagen. MMP-9 expression correlates with collagen misorientation, particularly desmoplasia.

TGF-b is known to increase MMP-9 production in a variety of cell types, most likely through a process that interferes with protein synthesis, leading to increased stability of MMP-9 mRNA.

On the other hand, increased MMP-9 is able to cleave latent TGF-b, leading to its activation. MMP-9 may indirectly participate in the fibrotic response through activation of TGF-b, a possible fibrogenic factor [11, p. 117].

MMP-13, also known as collagenase 3, has broad substrate specificity. Enzyme expression is enhanced under conditions that promote endothelial cell growth and vascular differentiation. The association of MMP-13 overexpression with non-healing wounds has been demonstrated using chronic skin ulcers as an example.

MMP-13 is localized in the plasma membrane of the affected endothelial cell layer, and NO leads to the release of MMP-13, which in turn is involved in epithelial tissue remodeling. MMP-13 is involved in wound healing in many tissues, but the role of MMP-13 in endothelial cell migration has not been fully determined [11, p. 118].

Based on the above, it can be concluded that inhibition or expression of MMPs directly affects the quality of remodeling of the intercellular matrix and leads to the formation of post-acne scar defects. Understanding the biochemical and pathophysiological processes of acne scar formation allows us to combat them at the early stages of their formation.

Correction of cicatricial changes

If we talk about already formed cicatricial changes, then, according to literature, there are three main types of atrophic post-acne scars: V-shaped (Icepick), M-shaped (Rolling), U-shaped (Boxcar). Since post-acne scars can be complicated by dyschromic, stagnant changes, it is best to use various techniques, as well as their combinations, when correcting post-acne.

To correct cicatricial changes in the skin in the post-acne stage, PRP therapy, mesotherapy, chemical peels, mechanical dermabrasion, laser ablation, RF technologies, injections of soft fillers, collagen preparations and course treatments including combinations of these methods are used. Combined techniques are the key to achieving optimal clinical results in patients with severe acne scars [12].

Fig. 1. The polycomponent nature of PRP allows us to say that it is a universal basic method of therapy at different stages of the disease, both in the early stages and at the stage of formation of post-acne processes. The method of preparing PRP in T-LAB PRP-tubes allows for the isolation of 1.95 Med DOSE (Dose of injected Platelets (billions)) per volume of up to 4.4 ml of plasma with a pure plasma level of 98.55 % [13].

PRP modulates and regulates the function of primary, secondary and tertiary growth factors, affecting all stages of regeneration simultaneously. The mentioned property distinguishes PRP growth factors from recombinant growth factors, each of which is responsible for a separate regeneration mechanism (table). [14]

Table. Regenerative mechanisms modulated by growth factors contained in PRP

Growth factor	Regeneration mechanism
PDGF	Activates proliferation and migration of mesenchymal cells, stimulates angiogenesis
IGF	Stimulates differentiation of young and stem cells, enhances metabolism
PDGF	Contains signal peptides. Produced by platelets and macrophages. Transforms cells that have the corresponding receptors, activates proliferation and migration of mesenchymal (osteogenic) cells
EGF	Stimulates the proliferation of fibro- and osteoblasts, the synthesis of fibronectin
FGF	They are produced by endothelial cells, macrophages, osteoblasts and platelets. 23 different FGFs are known in humans. The most important in the process of skin wound healing are fibroblast growth factor-2, fibroblast growth factor-7 and fibroblast growth factor-10. Fibroblast growth factor-2 activates the synthesis of matrix macromolecules, especially dermal glycosaminoglycans, hyaluronic acid and inhibits the synthesis of collagenase-1 in keratinocytes. Fibroblast growth factors 7 and 10 interact with fibroblast growth factor receptors-2-IIIb, which are found only on keratinocytes. *In vitro* studies have shown that fibroblast growth factors 7 and 10 stimulate keratinocyte proliferation and migration and play an important role in re-epithelialization
TGF-ß	The transforming growth factor "family" is produced by platelets and osteoblasts. They are found in large quantities in platelets. Increase the activity of some MMPs responsible for dermal matrix remodeling
DEGF	An enzyme that maintains the integrity of blood vessels. It has a stimulating effect on endothelial cells and has an angiogenic effect.

Fig. 2. Collost® gel contains type I collagen derived from bovine skin and is free of the cell pool and other components of dermal cells

Taking into account the data from scientific publications, monographs, articles, post-clinical studies and understanding the mechanisms of action of PRP and the Collost® dermal implant, we can confidently assume the synergism of their action.

By using the T-LAB PRP-tube system **(Fig. 1)** for the treatment of atrophic post-acne scars followed by implantation of Collost gel® **(Fig. 2)** , we can expect a more pronounced response to trauma during injections and active remodeling of the dermal matrix in the area of atrophic defects.

When administered intradermally, at the first stage Collost ® serves as a matrix for the formation of new tissue, creating optimal conditions for the migration and implantation of fibroblasts and improving their intercellular interactions.

A connective tissue capsule does not form at the product implantation site, since Collost ® does not provoke fibrosis, which is especially important for the treatment of cicatricial changes, namely post-acne.

When the cell pool of fibroblasts "populates" the collagen matrix under the influence of previously introduced plasma obtained using the T-LAB PRP-tube system and a powerful reparative process launched due to the release of growth factors, a pronounced remodeling of the intercellular matrix occurs with gradual degradation of the Collost® implant.

Clinical case

A 30-year-old female patient visited the clinic with complaints of multiple post-acne scars localized in the cheek area, formed during the course of acne.

History: acne of moderate severity, papulopustular form. The skin is porous, dense, turgor and elasticity are not reduced.

After the consultation, the patient was offered the following treatment tactics: injections of PRP obtained through the T-LAB PRP-tube system using the subcision technique in combination with the product Collost® 7 % within one procedure. The course consisted of three procedures with an interval of 4 weeks.

Protocol of the procedure

When isolating PRP using the T-LAB PRP-tube system, venous blood was collected in a volume of 10 ml. Anticoagulant - sodium citrate.

The use of tubes with sodium citrate is more appropriate than tubes with heparin, since sodium citrate, unlike heparin, does not affect platelet aggregation and, therefore, does not change the qualitative characteristics of the final product – PRP plasma.

Subcision is a painstaking but very effective method that a cosmetologist can use in their routine practice. Since deep post-acne scars are usually very tightly fused with the underlying tissues, aesthetic correction may be ineffective without this procedure.

The blood was centrifuged for 4 minutes at 2300 rpm. This centrifugation method produces 5 ml of PRP (platelet-rich plasma) and 3 ml of PPP (platelet-poor plasma). Two test tubes were used for one procedure.

PRP was injected into the lesions intradermally with subcision of scar elements. After releasing the tissue with a thin needle (cutting the fibrous strands), the product Collost® 7 % was injected directly under the scar defect in order to fill the resulting voids and initiate tissue restructuring [15].

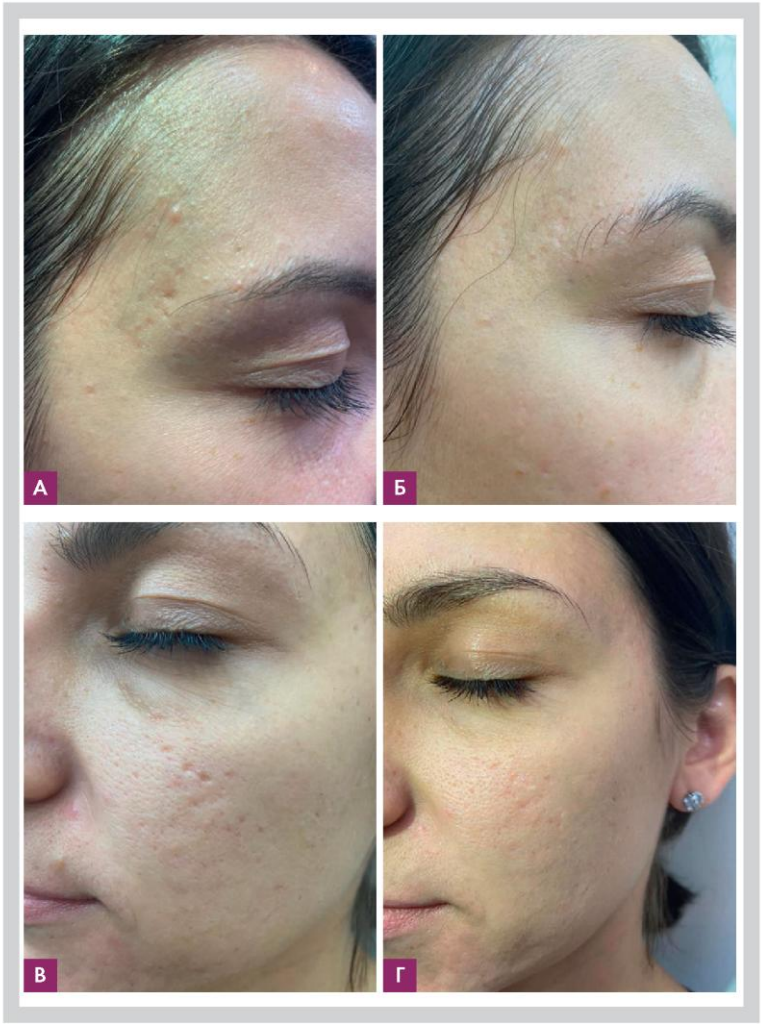

Fig. 3. Patient, 30 years old, before the course on the right (A) and left (B) and after two procedures on the right (B) and left (D)

After each injection procedure, the patient's skin requires time to fully recover. Accelerated treatment usually leads to complications. The standard protocol for using PRP involves injections once every 7–14 days, but since the subcision method is an additional traumatic factor, the recommended interval between the procedures performed using this technique should be at least 4 weeks and can be increased to 6 weeks in the case of extensive cicatricial changes correction [16]

The remaining volume of plasma was injected along the periphery of the foci of cicatricial changes using the microbolus technique to a depth of 4 mm and over the entire surface of the face using the deep dermal injection technique to a depth of up to 1.5 mm with maximum injection density into individual inflammatory elements.

The injection of PRP and Collost® 7 % was performed using local application anesthesia due to the painfulness of the procedure.

The remodeling effect of the product Collost® 7 % is prolonged in time, but the first results can be observed after two procedures **(Fig. 3)**.

And in conclusion

The results of the therapy clearly confirm the pronounced therapeutic activity of PRP in combination with the product Collost® in one procedure using the subcision method.

Due to the pathogenetic effect of the products on the links in the cicatricial deformation formation processes, the combined use of PRP and the product Collost® allows achieving significant clinical results already at the initial stage of therapy, two months after the start of the course.

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