The use of platelet-rich plasma in plastic and reconstructive surgery: the experience of the Henri Mondor Hospital

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Different adjuvant therapies have been developed in our department and used in several reconstructive surgery and esthetic medicine indications.
Over 8 years ago we began our collaboration with RegenLab SA (Switzerland) which has allowed us to develop different adjuvant therapies in several plastic and reconstructive indications as well as indications in esthetic medicine.
Platelet-rich plasma (PRP) is an autologous cell therapy containing numerous bioactive factors which are involved in wound healing and tissue repair. PRP preparation requires minimal handling to obtain the final product which can be used in several regenerative medicine indications in situ and provides the tools needed for tissue engineering.

The development of surgical adjuvants capable of triggering skin rejuvenation, enhancing wound healing, and reducing major surgical complications is one of the main goals of preclinical and clinical research in our department.

In our practice, autologous PRP, alone or in combination with other treatments, has been applied in plastic and reconstructive surgery. The indications can be divided in three main groups: reconstructive surgery, esthetic medicine, and in the field of gynecology for genital restoration.
The use of autologous PRP in plastic surgery and esthetic treatment of the face includes cervicofacial lifting, mesotherapy of the face by PRP alone or in combination with hyaluronic acid as an anti-aging treatment.
In the reconstruction of soft tissues (tissue damage resulting from burn injuries) and tissue augmentation (lifting, rejuvenation of the breasts, calf muscles and vagina), complementation of lipofilling with autologous PRP can improve the survival of fat grafts and give satisfactory results. Autologous PRP and PRP glue are often used to accelerate wound healing and improve the taking of skin grafts in cases of recalcitrant wounds (chronic and acute wounds).

CELLULAR MATRIX: REGENPRP AND HYALURONIC ACID IN ESTHETIC MEDICINE

Cellular Matrix: PRP in combination with hyaluronic acid in skin regeneration and facial rejuvenation

PRP injections take advantage of the regenerative and healing properties of blood platelets. Platelets can release the growth factors that are needed to induce the mechanisms for skin regeneration in the local environment. Plasma also contains factors essential for cell viability such as nutritive substances, vitamins, hormones, mineral salts and proteins that are required for the synthesis of a new extracellular matrix (Banihashemi and Nakhaeizadeh, 2014).

Hyaluronic acid (HA) is an essential component of the extracellular matrix and plays a major role in skin hydration. Its physiological functions are derived from its structural role in the extracellular matrix through its hygroscopic and rheologic properties which allow hydration and modulation of the cellular microenvironment. Hyaluronic acid has also a role to play in the cellular and tissue regeneration process where it facilitates the migration of a large number of different cell types to the damaged site, where it acts as an extracellular matrix scaffold that is able to support the proliferation and differentiation of cells during tissue regeneration. HA production decreases with age, which results in wrinkles, laxity, sagging, loss of elasticity and decreased hydration of the skin. In an in vitro model, it has been demonstrated that HA increases the proliferation and migration of fibroblasts. HA's viscoelasticity gives it lubrification properties. HA also regulates the hydration of tissues and their plasticity and protects cells from enzymatic attack. Finally, HA has an anti-radical and antioxidant role and protects cells from the deleterious effects of UV rays. HA seems to be a good scaffold for cellular therapy (Baspeyras et al., 2013; Stern and Maibach, 2008).

In a Phase I prospective clinical trial, we evaluated the efficacy and safety of PRP combined with hyaluronic acid injected into the dermis of the face compared with PRP alone or hyaluronic acid alone. The first results of this clinical study for the group treated with PRP combined with hyaluronic acid were published in the Journal of the American Academy of Dermatology and showed a significant improvement of the FACE-Q score at 6 months compared to baseline (44.3 ± 1.9 at baseline compared to 52 ± 3.17 at 6 months, P = 0.03). Biophysical measures performed using Cutomètre® showed a significant improvement for R5 (net elasticity) (P = 0.036) compared to the reference level (Figure 1). No adverse events were reported (Hersant et al., 2017).

A

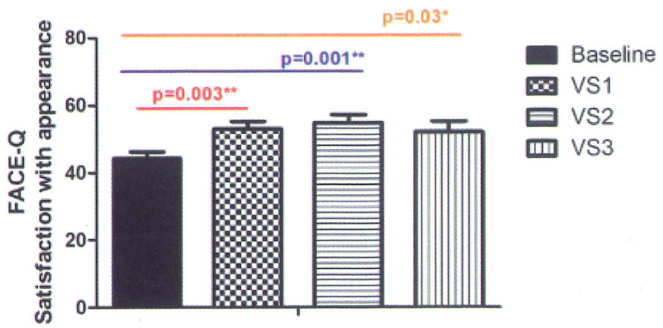
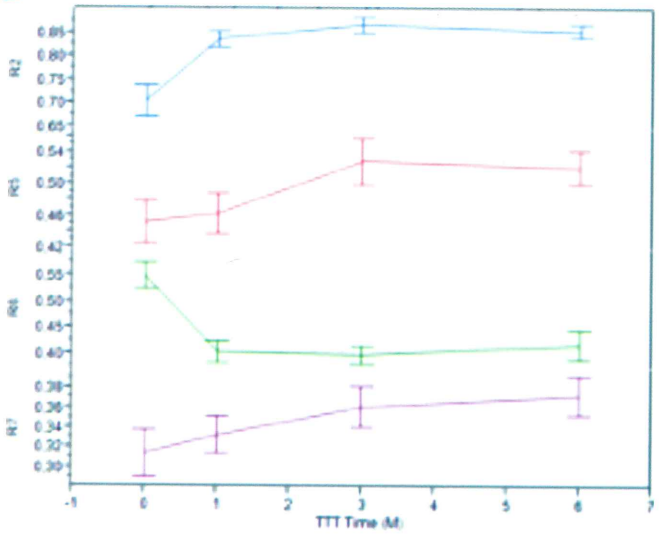


Figure 1: (A) Significant improvement in FACE-Q score and (B, on next page) skin elasticity parameters after 3 sessions of Cellular Matrix injections. VS1 (1 month), VS2 (3 months), VS3 (6 months). R2 gross elasticity in untreated skin, R5 net elasticity, R6 viscoelasticity, R7: immediate retraction/maximal deformation: its increase demonstrates an improvement in skin elasticity.

B



PRECLINICAL RESEARCH ON THE ROLE OF PLATELET-RICH PLASMA (PRP) AS AN ENHANCER OF ADIPOSE-DERIVED MESENCHYMAL STEM CELLS USED FOR CELL THERAPY

We have developed different basic research projects in our department in collaboration with teams from the National Institute of Health and Biomedical Research (INSERM) with a focus on the optimization of cell therapy and the development of new treatments for skin regeneration using adipose-derived mesenchymal stem cells (MSC) and PRP in a murine wound healing model to better understand the biological mechanisms.

Our first step, was to develop a murine wound healing model to evaluate the regenerative potential of MSC in combination with PRP and to study the biological mechanisms involved in the enhancement of MSC function by PRP, in particular the paracrine function and the survival of MSC, using in vivo and in vitro approaches.
The results obtained showed that treatment with an injection of MSC and PRP significantly improved skin regeneration at a macroscopic and functional level compared to treatment by either MSC or PRP alone. The time needed for healing was also significantly reduced by the combination of MSC and PRP compared to either alone. We were able to show that this clinical enhancement was due, at least in part, to modifications of the MSC secretome induced by PRP. In fact, MSC grafted in damaged tissue in the presence of PRP are able to secrete 24 to 45 times more VEGF at day 1, day 3 and day 30 and 100 times more IL-6 at day 1, day 3 and day 7 compared to MSC grafted alone. It was interesting to note that PRP did not have an effect on the MSC secretome when the MSC were grafted in healthy tissue. Moreover, we also demonstrated that PRP favored the survival of the MSC grafts in the damaged tissue without promoting their proliferation (no expression of the Ki65 marker). The in vivo results were partially validated in vitro since we show that PRP (20%) was able to induce expression of VEGF and IL-6 in MSC in culture.

Another research interest focuses on studying the role as well as the use of PRP for optimizing cell therapy in plastic surgery indications. Thus, we sought to develop the use of cell therapy in regenerative medicine techniques. To optimize our protocols, we used activated PRP which may increase the viability of adipocytes and adipose-derived MSC after grafting. We studied in an animal model the benefit of adding activated PRP to the transplanted adipose tissue in vivo and in vitro and its effect on the proliferation and differentiation of MSC. In a second project, we analyzed the effect of the combination of PRP with adipose-derived MSC in skin healing in a murine model and analyzed in vivo and in vitro the effect of PRP on survival and the paracrine function of MSC, in particular the pro-angiogenic and chemoattractant potential.
The results of these studies showed that PRP has a beneficial role in the survival of adipocytes during autologous fat grafting, in part due to the pro-angiogenic factors present in PRP, but also due to the ability of PRP to promote the proliferation of adipose-derived stem cells in the injected fat graft. Moreover, it seemed that the PRP effect was enhanced when activated by calcium chloride. Thus, the release of the growth factors can be done slowly and gradually in order to better support the transplanted adipocyte cells during the critical first week period (Hersant et al., 2018a).

In addition, cell therapy consisting of an injection of PRP with adipose-derived MSC seemed to improve skin healing in a murine model. We evaluated the ability of a treatment consisting of RegenPRP combined with adipose-derived MSC to repair the skin compared to either treatment alone or a saline solution in mice presenting with four lesions. Wound healing was measured on days 3, 7 and 10. We also examined in vitro and in vivo if PRP was able to modify the pro-angiogenic properties of MSC, their survival and their proliferation. The results obtained showed that PRP improved the efficacy of the MSC graft by accelerating the wound healing process and improving the elasticity of the newly generated skin. We discovered that, in vitro, the treatment with PRP stimulated, in a dose-dependent manner, the pro-angiogenic potential of the MSC due to increased secretion of soluble factors like VEGF and SDF-1. Moreover, PRP treatment increased the survival and proliferation of the MSC cultivated in vitro. These effects were accompanied by an alteration in the energy metabolism of the MSC such as the rate of oxygen uptake and mitochondrial ATP production. Several observations were made in vivo following administration of a combination of PPR and MSC in the wounds of mice. In conclusion, our study supported the fact the PRP used in combination with MSC could be a safe alternative to improve and accelerate wound healing (Hersant et al., 2019).

CONCLUSION

PRP continues to attract the attention of surgeons and doctors because of its clinical potential. In the field of plastic surgery, PRP is used as a complementary therapy for diverse clinical indications. In France, the competent health authorities have banned the use of PRP in esthetic medicine because of the inconclusive findings from clinical studies and a lack of long-term follow-up in relation to the safety of the patients.

To this day, no study has reported the long-term effects of PRP in terms of side effects such as cancer- or other disease-related pathologies directly linked to treatment with PRP. We have therefore conducted a retrospective study and reported all the data relating to the safety of the use of RegenPRP either alone or in combination with other biological or chemical agents in different plastic surgery and esthetic indications. Information on short-term and long-term complications was gathered via a consultation and a questionnaire given to patients. The data from all the patients treated with RegenPRP over the last six years, i.e., from January 2013 to January 2019, were collected. All patients were satisfied with the treatment. No serious complications have been reported following the use of RegenPRP.

We therefore consider that RegenPRP can be safely used in different plastic surgery and esthetic indications as well as for facial and vaginal rejuvenation.

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Correction of Age-Related Changes with Autologous Injection of Platelet-Rich Plasma and Hyaluronic Acid Cellular Matrix in Combination with Cosmetology Equipment

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RELEVANCE

Nowadays, cosmetologists possess a large arsenal of tools, such as various medical equipment, injection methods, and cosmeceuticals. For cosmetologists, age-related skin changes pose a difficult task that cannot be solved even with the most advanced technologies when used alone. Therefore, methods that when used in combination affect different cellular mechanisms relating to skin cells and the extracellular matrix are very relevant. The main goal of any combination is to mutually enhance the correction effect and reduce any unwanted reactions. In order to achieve this, it is necessary to improve the condition of the skin in many respects: from surface characteristics to a profound change in the activity of cellular organelles and in tissue structure. We can minimize undesirable effects by including methods that support reparative processes through improving microcirculation and regeneration of tissues. The use of medical equipment for cosmetology is based on the induction of controlled micro-trauma to the skin through various physical methods, among them:

- Laser radiation with different wavelengths, energy, shape and frequency of pulses
- Focused ultrasound
- Electric currents with different characteristics, energy, shape and frequency of pulses
- Broadband light

Common to all equipment-based methods is the fact that the controlled trauma upsets the physiological balance of skin tissue, which is followed by restructuring. In response, processes that return the tissue to the balanced state are triggered. Under the influence of physical factors underlying equipment-based methods, a greater number of epidermis, dermis, and subcutaneous tissue cells transform to the active state. This is considered as a kind of training, which, as in the gym, leads to improvements in structural and functional levels. Just like after physical exertion in the gym (remember adequate training and well-adjusted sports nutrition), recovery processes in the tissues need support for successful adaptation after becoming unbalanced by the use of the equipment. Only if there is an optimal ratio between the stress factor, which in this case is the action of the equipment, adaptive abilities and structural resources of the tissue itself, can we obtain the desired effect from the use of the equipment (Deev et al., 2014). To improve and optimize the skin's ability to regenerate and restore itself, biological injection methods are used, in particular, the therapy with autologous platelet-rich plasma (PRP-Platelet Rich Plasma, PRP-therapy). Also, preparations based on hyaluronic acid have been used for these purposes for a long time. Today, the combined approach is also available to us for use—Cellular Matrix® for intradermal injections. This is a medical device, patented and registered in the Russian Federation for intradermal injections, that contains patient's autologous PRP together with a particular form of hyaluronic acid.

EQUIPMENT-BASED METHODS

Treatment methods based on the use of medical equipment for cosmetology are constantly developing. Today, they provide us with various physical factors for correcting the signs of skin aging, such as electromagnetic radiofrequency (RF) treatment (Franco et al., 2010). The mechanisms involved in RF lifting are primarily based on the thermal effect and aseptic inflammation at the site of the controlled micro-trauma. Their depth directly correlates with the frequency of the alternating current passing through the skin — the lower the RF, the more pronounced the effect (Sadick and Makino, 2004). The RF micro-needling

method delivers energy to deeper layers of the skin compared to non-ablative fractional photothermolysis (Fig. 1). The depth of needle insertion is controllable and can vary depending on the required degree of exposure and the anatomical zone, which is an important distinguishing characteristic of the method (Carruthers, 2001). Combined mechanical and radio-frequency actions induce neocollagenesis in the dermis (Friedman and Gilead, 2007).

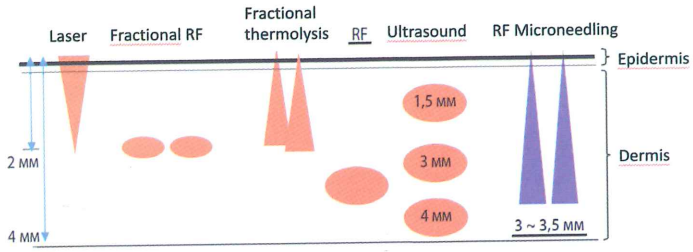


Fig. 1: RF Microneedling delivers RF energy from the epidermis to the deep dermis along the entire length of the needle, inducing maximum collagen renewal

PRP IN COMBINATION WITH MEDICAL EQUIPMENT TREATMENT

Effective rejuvenation methods also include PRP therapy. Its effectiveness is based on initiating reparative and trophic processes which are activated by various paracrine factors within the platelets (Redaelli et al., 2010). Platelet-rich plasma is a natural agent (autologous medication); its absolute biocompatibility is beyond doubt, as it is obtained from the patient's own blood. PRP has a high wound healing and regenerative potential because it is a concentrate of the main bioactive substances involved in different stages of the recovery process. The effect of PRP follows degranulation of the platelet alpha granule content — glycoproteins and growth factors: platelet growth factor, transforming growth factor beta, fibroblast growth factor, vascular endothelial growth factor, and epidermal growth factor. Notably, the biological "cocktail" secreted by platelets has the ideal physiological proportion of growth factors. Upon contact with a surface, whose structure is different from the vascular endothelium, and under the action of thrombin, platelets activate, transform, and produce pseudopods that fix them to the fibrin network. Platelets are involved in all stages of the physiological healing process following any injury; a similar mechanism is observed with PRP therapy. Under the action of growth factors, dermal fibroblasts synthesize collagen, elastin, and a non-fibrous matrix, which strengthen and lift the skin. Also, new capillaries are formed (angiogenesis) and the epidermis is renewed (Rappl, 2011; Redaelli et al., 2010; Smith et al., 2007). For a clinically significant effect, it is important to have a sufficient number of viable platelets, which, after activation by patient's own thrombin, release growth factors and other bioactive substances into the surrounding intercellular space. Growth factors interact with platelet membranes and transform to the active state, thus becoming bioavailable. If, however, platelets simply undergo destruction without being activated by thrombin, growth factors remain unactivated and unable to provide a the biological effect.

The main indications for PRP therapy consist of skin aging in the face and body, acne and diffuse hair loss. These are the main cosmetic problems that we have gained significant practical experience in, which includes knowledge of the physiological and functional mechanisms behind the therapeutic effect of PRP.

The beneficial effect of using correctly prepared PRP technology for skin treatment has been developed in laboratory and during clinical studies. Key conditions need to be respected:

- platelet concentration should be 1.5–2.5 times higher than in whole blood. Notably, concentrations 2.5–3.0 times higher lead to mutual inhibition of platelets and marked decrease in the release of growth factors (Yamaguchi et al., 2012);
- absence of heparin, which blocks platelets' ability to aggregate on fibrin, and therefore blocks the physiological process of activation and release of growth factors;
- cells must not be mechanically destroyed during preparation, since growth factors become biologically active only when they are pre-formed while passing through membranes of unbroken, functionally active platelets;

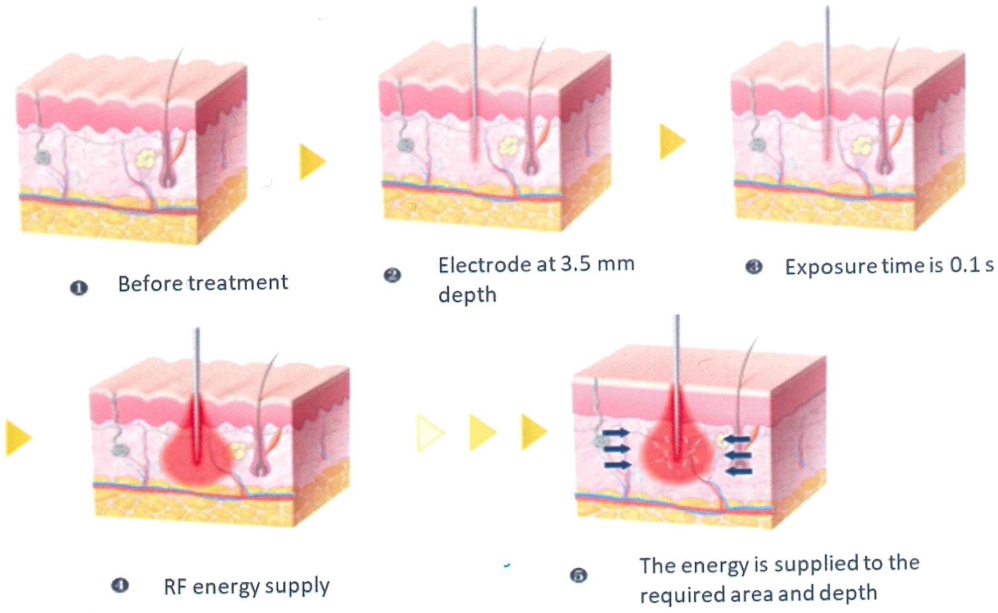


Fig. 2. The mechanism of action of RF micro-needles.

- presence of additional matrix: autologous fibrin and / or hyaluronic acid;
- reduced proportion of granulocytes, which have pro-inflammatory activity;
- absence of red blood cells;
- presence of monocytes, which contain a few growth factors and increase the effectiveness of the preparation through support of non-specific immunity.

The combination of RF lifting and PRP therapy has shown high efficiency in aesthetic medicine for correcting signs of skin aging (Alenichev, 2018).

Injectable preparations based on hyaluronic acid (HA) are well-known among cosmetologists. Hyaluronic acid exhibits various positive biological effects following its introduction into tissues. This depends on its molecular weight, concentration, its cross-linking level, and few other factors. In the skin, HA molecules larger than 500 kDa create conditions for cell migration and proliferation and maintain homeostatic equilibrium, being a sort of resource for self-restoration and adaptation. When damage and subsequent inflammation affects the tissue, HA undergoes biodegradation forming medium- and low-molecular fragments that perform the functions of signaling molecules.

The medical device Cellular Matrix, which we selected for the complex therapy, combines the patient's autologous PRP with hyaluronic acid (HA). The unstabilized hyaluronic acid contained in the tube acts as a scaffold-like structural element, which is similar to the skin, while the PRP provides the reparative and regenerative effect. The presence of hyaluronic acid exactly at the time of fibrin polymerization allows for the formation of three-dimensional spacious, tightly packed, structures in which platelets are dispersed. These structures are colonized by fibroblasts, thereby providing increased synthesis of extracellular matrix components and organized reconstruction of the dermis. When the preparation, containing patient's autologous PRP and HA is injected into target tissues, a biologically enriched network is formed — a matrix that facilitates cell migration and proliferation, improves hydrodynamics in the tissue, and provides distribution of trace elements, metabolites, and hormones (Turzi, 2018). The cellular matrix acts as a reservoir for activation of growth factors and increases their residence time in the tissue as compared to PRP alone. The synergy of the biological effects of PRP and hyaluronic acid in a single cellular matrix is important, since the clinical effects are much more pronounced as compared to the use of either of these components alone.

MATERIALS AND METHODS

In the Clinical Institute of Anti-Aging Medicine (Moscow), for the treatment of the signs of skin aging specifically in the face and neck areas, we use a protocol combining the cellular technology and micro-needling RF treatment with an electrosurgical radiofrequency device.

The method is based on the ability to obtain a balanced combination of hyaluronic acid, produced through fermentation, and PRP in one tube. Cellular Matrix consists of the patient's autologous PRP combined with unstabilized hyaluronic acid. It allows for a one-step preparation of biologically identical material for immediate use in a completely closed sterile system. With this technology, it is possible to extract at least 80-90% of platelets from whole blood (with complete removal of red blood cells) in a mixture containing 2 ml of a 2% unstabilized HA solution. Platelets and monocytes extracted using this method retain their full viability and functional activity and are able to secrete growth factors (Turzi, 2018). The use of this product results in a physiologically orchestrated activation of regenerative processes due to the action of platelet growth factors and interleukins, whereas the hyaluronic acid contained in the preparation provides the tissue with structural material to restore the non-fibrous matrix of the dermis, optimizes hydration, and exhibits a "remodeling" effect, thus contributing to the filling of wrinkles and folds. It simultaneously provides 3D-modeling of tissues, correction of visual skin defects, and regeneration and reorganization of the skin's microstructure.

Radio-frequency therapy was conducted using an RF device designed for minimally invasive fractional bipolar RF therapy. Energy of the RF device has a direct effect (heating) on the epidermis and dermis at a depth of 0.5 to 3.5 mm. There are 25 uninsulated needles on the head. Uninsulated micro-electrodes deliver RF energy without loss, directly to the selected zone, where water evaporates from the micro-thermal zone and stimulation occurs (Fig. 3).

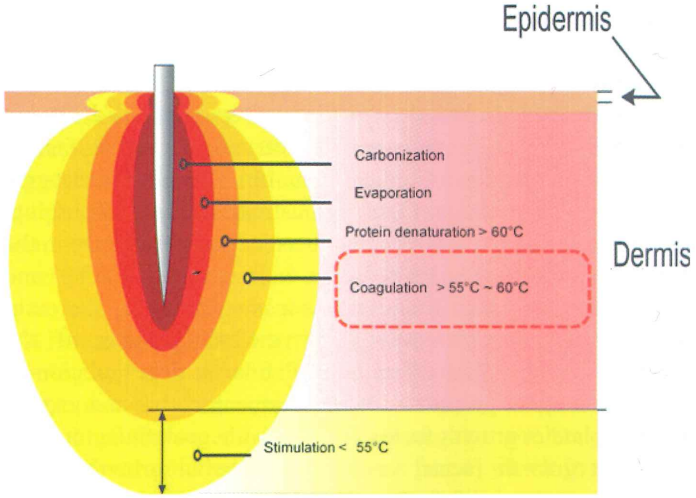


Fig. 3. The effect of RF microneedling on tissue.

For facial and body skin rejuvenation, a course of treatment included three treatment cycles, which were performed one month apart according to the following scheme: the micro-needling RF lifting treatment was provided; then, a week later, injection of Cellular Matrix (PRP in combination with HA) was provided. Detailed practical aspects of injecting PRP and Cellular Matrix are described in the contribution by Dr Sharypova and colleagues. After a three-week break, this cycle was repeated again. In total, there were three cycles of the combined therapy according to the developed scheme. It is important that in this combined treatment for age-related skin changes, the recommended monthly intervals are followed both between the equipment- and injection-based treatments. Also, the injections of Cellular Matrix were performed shortly after the mechanical stimulation of the dermis. This contributed to quick restoration of the skin, which had undergone therapeutic controlled skin trauma, as well as to additional activation of neocollagenesis and restoration of microcirculation.

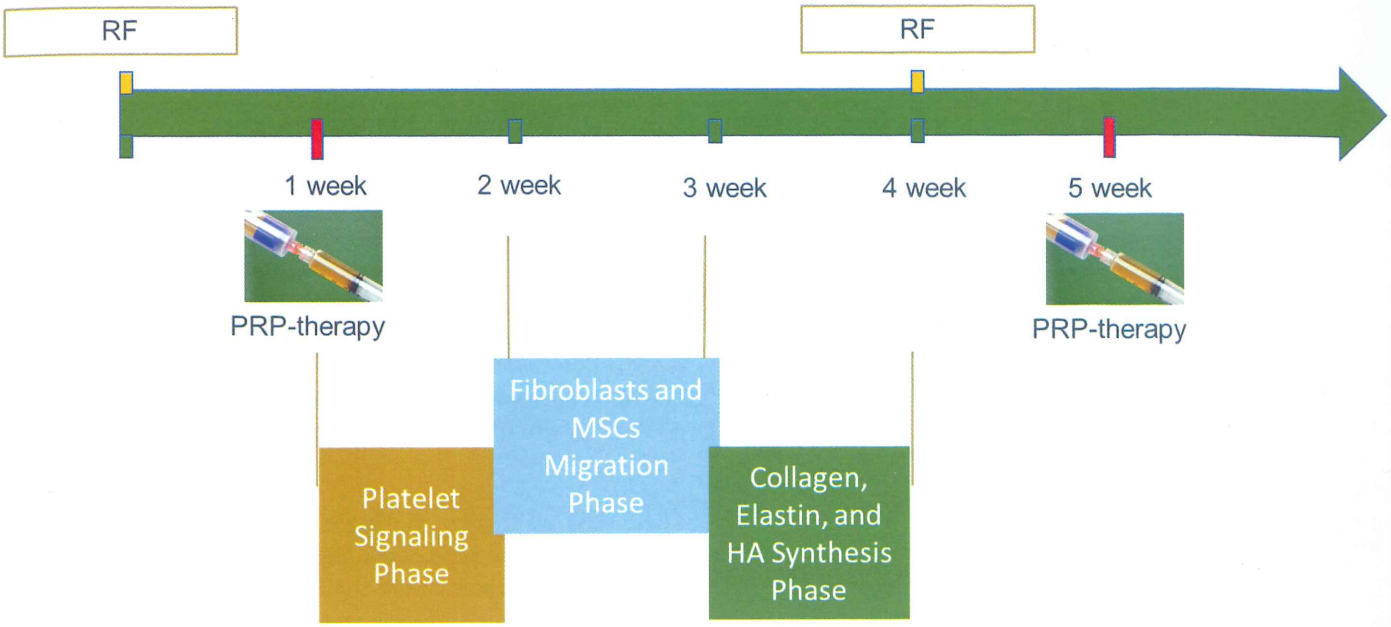


Fig. 4 Scheme for Combining PRP Therapy with Equipment-Based Treatment

INCLUSION CRITERIA

This study included females and males, aged 40-70 years. Patients must have signed an informed consent form to be included in the study. Moreover, patients were required to have no hypersensitivity to HA and no contraindications for RF therapy.

EXCLUSION CRITERIA

Skin diseases in the active phase, acute herpes and other infections on the skin, exacerbation of general infectious diseases or chronic somatic diseases, mental illness, usage of antidepressants or medications that affect the central nervous system, epilepsy, cancer.

We observed 20 patients with signs of facial skin aging, aged 40 to 70, who underwent the combined therapy according to the developed protocol.

To assess the stratum corneum hydration before and after the treatment course, corneometry was performed using Corneometer CM 825. Transepidermal water loss (TEWL) was evaluated before and after the treatment using Tewameter TM300 vaporimeter. The effectiveness of the aesthetic correction of facial skin was evaluated with visual analogue scales, photography and the quality of life evaluation.

The results were compared depending on age: a group of patients under 49 y.o. (n=10) and a group of patients above 50 y.o. (n=10).

RESULTS

Comparative assessment of skin functional parameters (hydration and TEWL) showed promising results of the combined therapy with Cellular Matrix and RF lifting (Table 1). Indeed, hydration of the stratum corneum after the combined therapy increased by 1.7 times in the patients under 49 y.o. and by 2.1 times in the patients above 50 y.o., while TEWL decreased by 2.8 times in the group under 49 y.o. and by 2.2 times in the group above 50 y.o.

Cellular Matrix + RF					
Parameters	Under 49 y.o.		Probability	Above 50 y.o.	
	before treatment	after treatment		before treatment	after treatment
Hydration	51.1 ± 2.8	85.8 ± 3.6	P<0.001	38.7 ± 3.4	79.9 ± 4.2
TEWL	23.7 ± 1.6	8.5 ± 1.9	P<0.01	28.1 ± 2.6	12.9 ± 1.7

Table 1: Skin Hydration and TEWL on patients having Combined Therapy with Fractional RF Micro-Needling and Cellular Matrix injection.

Assessment with visual analogue scales showed a decrease in main signs of chronoaging in both groups (Fig. 5 and 6).

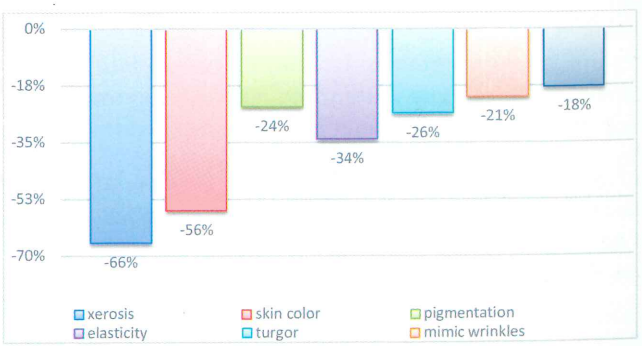


Fig. 5. Decrease in clinical signs of skin chronoaging following the combined method treatment, according to the VAS index (qualitative characteristics of the skin and the severity of wrinkles) in patients under 49 y.o.

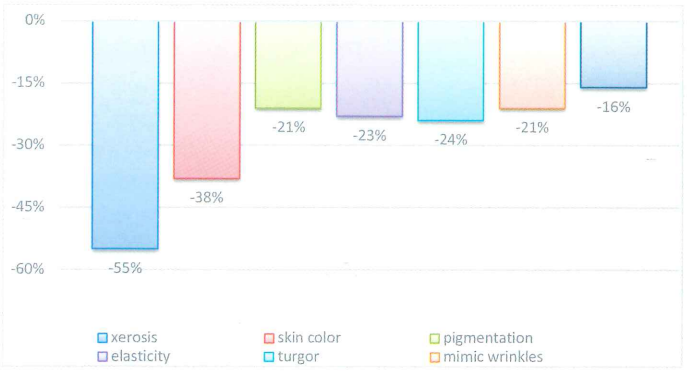


Fig. 6. Decrease in clinical signs of skin chronoaging following the combined method treatment according to the VAS index (qualitative characteristics of the skin and the severity of wrinkles) in patients above 50 y.o.

The improvements in qualitative characteristics of the skin, as well as the reduced severity of mimic and gravitational wrinkles, contributed to improving the patients' quality of life. On average, the DLQI index improved by 79.9% in the patients under 49 y.o.: from 14.5 ± 0.7 to 2.9 ± 0.8 ($p<0.01$) (Fig.7), and by 75.4% in the patients above 50 y.o.: from 16.2 ± 0.9 to 4.0 ± 0.5 points ($p<0.01$) (Fig. 7).

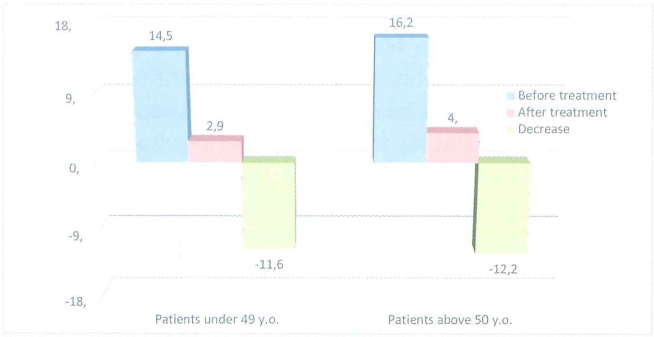


Fig. 7. Mean scores of the Dermatology Life Quality Index (DLQI) following combined method treatment for correcting signs of facial skin aging (y axis: the median values, at $p<0.01$ according to the Mann-Whitney criterion).

Patients were also assessed for treatment satisfaction (effectiveness, comfort, and safety of the treatment); it reflects the patient's opinion and is based on comparisons between the expected and received effects when compared to other methods used for similar indications. The satisfaction index according to the researcher (doctor) and the patient was statistically comparable in all aspects. The overall assessment by the patients was as follows: 90% of the patients, regardless of age, reported good / very good efficacy and comfort of the combined method (Fig. 8).

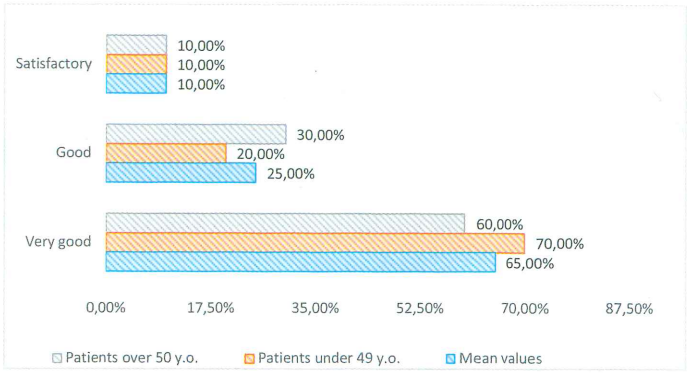
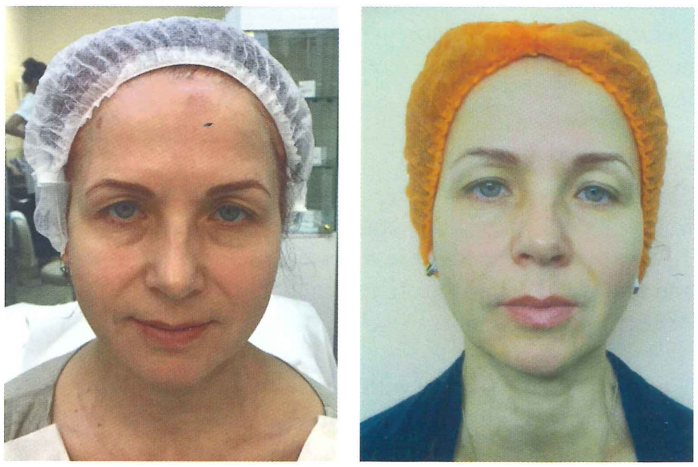


Fig. 8. Patient questionnaire data on the effectiveness / comfort / safety of the combined method for correcting signs of facial skin aging.

These data were fully consistent with the data obtained from physicians: 100% physicians were satisfied or very satisfied with the effectiveness of the combined method. Noticeably, the rejuvenation effect from the treatment lasted for up to 1.5–2 years in most patients.

The dermatoscopy and photography data also confirmed the clinical effectiveness of the combined therapy protocol (Fig. 9).

Before the combined treatment, patient 1, 44 y.o.
After the combined treatment, patient 1, 44 y.o.



Before the combined treatment, patient 2, 53 y.o.
After the combined treatment, patient 2, 53 y.o.

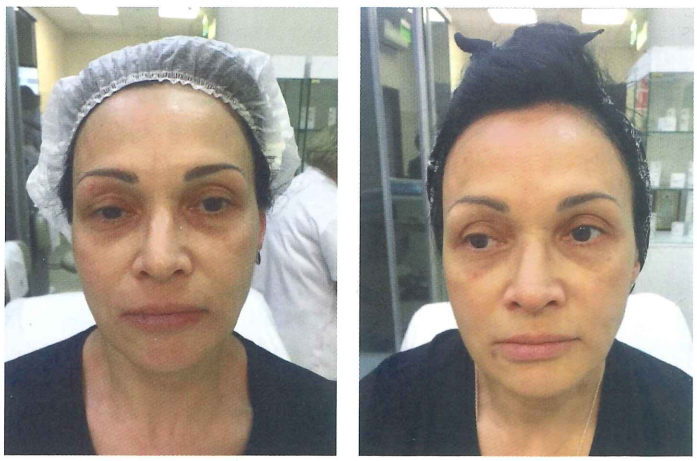


Fig. 9. Clinical examples of using the combined method (RF lifting and cellular matrix) for correcting age-related skin changes