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Comprehensive approach to correct involutional-dystrophic skin changes in women

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ABSTRACT

Skin aging is one of the most urgent problems that is not being studied today. The study involved 107 women with signs of age-related changes in the skin of the face aged 25 to 60 years. They were treated according to our autor's technology neofibrolifting - autologous fibroblasts transplantation into the pretreated with platelet-rich plasma skin and ablative fractional photothermolysis procedure. The developed neofibrolifting technology allows reconstructing authentic skin structure, improving its appearance and immunity.

Key words: Fibroblasts; platelet-rich plasma; aging; skin; skin wrinklings; laser ablation.

Introduction

Skin aging is one of the most urgent problems that is being studied today not only by dermatologists and cosmetologists, but also by specialists in the field of molecular biology, pathomorphology, pathophysiology, genetics and other areas of medicine. This is a complex, multi-factor process that is being actively studied today. More and more data is being accumulated on the role of immune system involution in general skin aging, including its natural and Adaptive Immunity.[1-6] Immuno-senescent atrophic and dystrophic phenomena in the skin are manifested by a pronounced decrease in the number of dermal fibroblasts and suppression of their functional activity. As a result, there is a violation of the normal process of recovery of dermal fibroblasts, the formation of the intercellular matrix of the dermis, which leads to the appearance of pronounced involutional signs.[1,7-9] Among them are thinning of the skin, dryness, flabbiness, loss of elasticity, the formation of wrinkles of varying degrees of expression, etc.

Despite the wide variety of methods for correcting age-related skin changes, all of them either do not allow achieving the desired long-term result, or they are associated with a large number of various complications.

Research purpose

The aim of this study is increasing the effectiveness of methods for correcting involutional-dystrophic changes in the skin in women with physiological aging by developing a complex technique of neofibrolifting – transplantation of autologous

**Running Head:*
Correction of involutional-dystrophic skin changes in women
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dermal fibroblasts into the skin with the previous introduction of platelet-rich plasma and activation of regeneration processes, renewal of the microstructure skin due to controlled inflammation created by fractional photothermolysis.

Materials and Methods

The study involved 107 women with signs of age-related changes in the skin of the face aged 25 to 60 years. All patients were thoroughly familiarized with the course of the study and signed the relevant informed consent. The criterion for inclusion in the study was the presence of involutional signs of skin, age from 25 to 60 years. The exclusion criteria were skin diseases in the active phase, herpetic and other infectious processes on the skin in the acute phase, General infectious diseases, chronic somatic diseases in the acute stage, mental diseases, epilepsy, tendency to form keloid scars, oncological diseases, pregnancy, lactation, pathology of the blood clotting system, connective tissue diseases.

All patients were divided into groups depending on age: 1st (25-35 years), 2nd (36-45), 3rd (46-55) and 4th (56 and older). The comparison group consisted of 22 women aged 25-35 years without visual signs of Chrono - and photoaging, who were examined for immunological and structural-functional skin indicators.

The study of the structural and functional state of the skin was carried out by instrumental methods. To assess structural changes in the skin, the method of ultrasound dermascanning was used using a portable high – frequency ultrasound device "DUB-Digital Ultraschall Bildsystemtpm" and DUB-SkinScan ver software.3.2 (Germany). The moisture content in the epidermis was estimated by corneometry, which is based on measuring the electrical capacitance of a dielectric medium. The state of the barrier function of the epidermis was studied by measuring TEVV. For these studies, a multi Skin Test center® MC 1000 diagnostic combine (Courage+Khazaka electronic GmbH, Germany) was used. Blood flow determination was performed by ultrasound Doppler scanning using the Minimax-Doppler-K device (Russia). OSC was measured in the skin of the forehead and cheek (ML/s/cm3; Figure 1, 2, 3).

Skin sampling for cultivation and production of fibroblasts, as well as immunological studies, was carried out by biopsy. Tissue samples were placed in a sterile container and transferred to a biotechnological laboratory equipped according to the requirements of Good Manufacturing Practice (GMP). Flow cytofluorimetry and a general protocol for immunophenotyping using primary antibody conjugates were used to prepare cell suspensions and conduct immunological studies. All measurements were performed on the FACSCalibur flow cytofluorimetry device manufactured by Becton Dikinson with

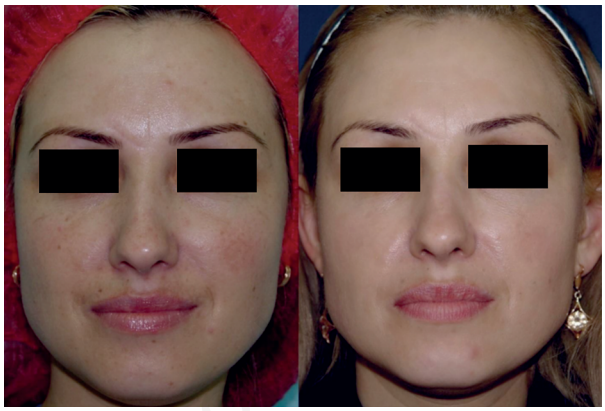

Figure 1. Patient N., 32 years old, before and after treatment.

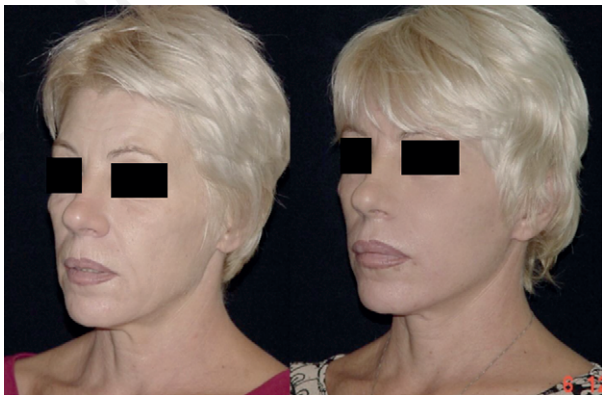

Figure 2. Patient I, 50 years old, before and after treatment.

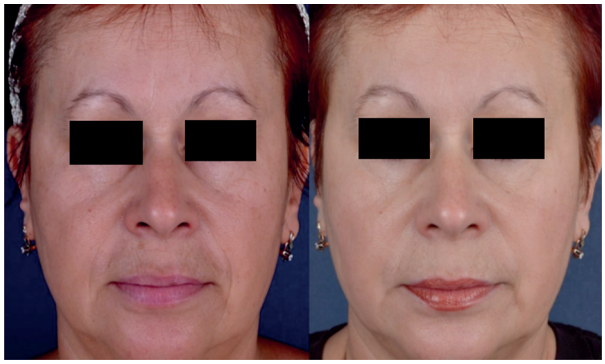

Figure 3. Patient V., 56 years old, before and after treatment.

antibody kits for cell phenotyping BD Multitest IMK Kit (BD Multitest™ CD3FITC/CD8PE/CD45PerCP/CD4APC reagent, BD Multitest CD3FITC/CD16PE+CD56PE/CD45 PerCP/CD19APC reagent) in compliance with the manufacturer's recommendations for use equipment and reagents. The harvest Smart PReP2 automatic centrifuge (USA) was used to produce Platelet-Rich Plasma (PRP).

A protocol for the study and treatment of patients was developed. After clinical, laboratory and instrumental examinations, 107 patients of different ages underwent a skin biopsy to culture dermal fibroblasts and study immunological parameters. After 2 weeks, the PRP injection procedure was performed in the form of intradermal injections (14 ML) and ablative fractional photothermolysis procedure (erbium laser Dermablate effect Asclepion; Germany), which, by creating controlled inflammation, enhances regeneration processes, renews the microstructure of the skin and improves its metabolism[5] and creates the most favorable conditions for the subsequent introduction of dermal autofibroblasts. After another 2 years, a skin biopsy was performed for immunological studies and autofibroblast transplantation in the form of intradermal injections (60 million cells), instrumental studies.

Another 2 weeks after the fibroblast transplant, the procedure for taking a skin biopsy was performed for immunological studies, and instrumental studies were performed. 6 and 12 months after fibroblast autotransplantation, the procedure for taking a skin biopsy for immunological studies and instrumental studies was performed by 32 women of different age groups [1st (n=8), 2nd (N=7), 3rd (n=9), 4th (n=8)].

The results obtained were processed statistically using the Statistica 8.0 software package (StatSoft, Inc.) with the calculation of the median (me) and interquartile span (25-75 %). Comparison between two independent groups with a different data distribution than normal was performed using the Mann-Whitney U-test. The results were considered statistically significant at 95 % ($p<0.05$).

Results and Discussion

In the 1st group of women (25-35 years), early age-related changes were determined, primarily characterized by the presence of facial and superficial static wrinkles (Class 2A). The 2nd group of patients (36-45 years old) was dominated by deep static wrinkles with initial manifestations of gravitational ptosis (Class 2B). Individuals of the 3rd Group (46-55 years old) were found to have deep static wrinkles and gravitational ptosis of the 1st-2nd degree (Class 3A). In the 4th group of women (56 years and older), deep facial and static wrinkles, gravity ptosis of the 3rd degree (grades 3B, 3B) were noted.

The thickness of the epidermis in patients of Groups 1 and 2 did not significantly differ from those of practically healthy individuals, and in patients 46 years and older, a significant decrease in the thickness of the epidermis was observed in relation to GP: in Group 3-by 22 % ($p<0.05$), in Group 4-by 21 % ($p<0.05$). The thickness of the dermis in women aged 25-35 years was almost the same as that of GP. In older age groups, the indicator significantly decreased in relation to GP [in Group 2-by 22 % ($p<0.05$), group 3 – by 10 % ($p<0.05$), Group 4-by 24 % ($p<0.05$)] and group 1 ($p<0.05$). The acoustic density of the skin in Group 1 was almost the same as that of the GP. In the 2nd and 3rd groups, an unreliable decrease in this parameter was found. Only group 4 showed a 22% decrease in skin acoustic density ($p<0.05$) compared to GP and Group 1.

In Group 1, the results of corneometry practically did not differ from the values in practically healthy individuals. In other age groups, a decrease in the moisture content in the epidermis was determined relative to the GP data [in Group 2-by 19 % ($p<0.001$), group 3 – by 30 % ($p<0.001$), Group 4-by 40 % ($p<0.001$)] and group 1 ($p<0.001$). The indicator of the 3rd Group statistically significantly decreased in relation to the 2nd ($p<0.001$). Even more epidermal moisture decreased in patients of the 4th group (56 years and older), significantly relative to the indicator of the 2nd and 3rd groups ($p<0.001$ and $p<0.05$, respectively).

In Group 2, TEVV did not significantly increase relative to GP levels, despite a significant decrease in epidermal thickness in this group. Probably, the condition of the epidermis is determined not only by the loss of moisture. In the two older age groups, especially in the 4th, the TEVV index significantly increased relative to the GP level [in the 3rd group-by 31 % ($p<0.001$), the 4th-by 65 % ($p<0.001$)], the 1st and 2nd groups ($p<0.01$). It was highest in the last age group (4th), in which the value significantly ($p<0.01$) exceeded the level of the 3rd Group.

Despite the rather clear visual manifestations of involutional changes, it is considered appropriate to support purely clinical observations with objective methods when conducting innovative studies. Among the latter, non-invasive instrumental methods for studying skin properties are widely recognized. It is important to note that the epidermis becomes thinner in areas of the skin where there are signs of blood flow suppression, and does not change in those where normal blood flow is present.[7]

OSC in the forehead area in the 2nd group of patients decreased relative to the level of GP, although unreliably. In Group 3, there was a significant decrease in the indicator by 22 % ($p<0.05$) relative to GP. In the oldest age group, Osh in the forehead area decreased by 72% relative to GP ($p<0.05$) and relative to other groups ($p<0.001$). When studying OSC in the cheek area, it turned out that it significantly decreased by 35 % ($p<0.01$) in the 2nd group compared to GP and 1st ($p<0.01$). In the future, there was an even greater decrease in Osh in the cheek area relative to GP [in Group 3-by 53 % ($p<0.01$), 4 – by 69 % ($p<0.001$)] and Groups 1 and 2 ($p<0.01$). As in the forehead area, a significant decrease in OCD in the cheek area was also determined in the 4th age group compared to all other groups ($p<0.001$).

Minimization of age-related structural and functional disorders is possible by replenishing the dermis with fibroblasts, introducing a certain number of functionally complete cells with greater synthetic and regulatory activity, and using neofibrolifting. In order to increase the clinical effectiveness of neofibrolifting, it was considered advisable to induce an inflammatory process in the skin before the introduction of fibroblasts by preliminary platelet injections, which ensures the appearance of pro-inflammatory cytokines and growth factors in the tissue, which significantly activate the proliferation and differentiation of fibroblasts and ablative fractional photothermolysis, which promotes the synthesis of new structural elements of the epidermis and dermis, reorganizes the surrounding tissue and creates favorable conditions for further introduction of fibroblasts. Under the influence of neofibrolifting, significant changes in indicators occurred. However, the thickness of the epidermis in Group 1 (25-35 years) did not differ from the value in GP and did not change during neofibrolifting (Table 1). In Group 2 (36-45 years), it did not differ from GP scores and increased by 17 % ($p<0.05$) relative to pre-treatment levels after fibroblast transplantation, but returned to baseline levels after 6 and 12 months. In patients aged 46-55 years before treatment, the thickness of the epidermis was 22% less than that of GP ($p<0.05$). In the specified group, the growth of this indicator by 20 % ($p<0.05$) relative to pre-treatment data occurred after PRP management and ablative fractional photothermolysis and it was at the same level as a result of the introduction of autofibroblasts immediately, after 6 and 12 months of follow-up. In patients of Group 4 (56 years and older), before treatment, the thickness of the epidermis was 21% less than in GP ($p<0.05$). Immediately after autofibroblast transplantation, the thickness of the epidermis also increased (by 19 %; $p<0.05$), but remained at the achieved level only until 6 months. The introduction of PRP in this group was ineffective.

Dermal thickness in the youngest group of patients (25-35 years) before treatment did not differ from the GP level and increased by 9% compared to the pre-treatment index ($p<0.05$) after PRP administration and ablative fractional photothermolysis, 11 % ($p<0.05$) – fibroblast transplants, 18% ($p<0.05$) – after 6 months of follow-up. After 12 months, the thickness of the dermis practically did not differ from the level before treatment. In the groups 36-45 and 46-55 years of age, the thickness of the dermis before treatment was less than that of GP, by 21% and 10 % ($p<0.05$), respectively. A significant increase in the indicator relative to the pre-treatment level in these groups was observed only 12 months after fibroblast administration: in Group 3 – by 28 % ($p<0.05$), in Group 4-by 19 % ($p<0.05$). In patients 56 years and older, the thickness of the dermis before treatment was 24% less than that of GP ($p<0.05$). The response to treatment in Group 4 was most pronounced: thickening of

Table 1. Thickness of the epidermis in patients of different age groups in the dynamics of treatment

Age groups	Statistical indicators	Thickness of the epidermis, microns					
		In the GP	Before treatment	During treatment after entering PRP and ablative fractional photothermolysis	Fibroblasts	After treatment via 6 months	12 months
2nd floor	Me 25-75% n	108.2 88.4-124.0 12	87.1 76.5-112.0 15	87.5 76.9-112.4 15	102,2# 91,6-127,1 15	98.1 92,7-101,6 7	95.6 90,2-99,1 7
3-тя	Me 25-75% n	108.2 88.4-124.0 12	84.7* 63.4-94.3 13	102.0# 80,7-111.6 13	103,.2# 81.9-112.8 13	103.6# 84.7-112.3 9	103.9# 85.0-112.6 9
4-та	Me 25-75% n	108.2 88.4-124.0 12	85.5* 73.4-102.1 12	83.4* 71.3-100.0 12	102.0# 89.9-118.6 12	105.6# 97.4-117.1 8	85.4* 77.2-96.9 8

*Note: *p<0.05 relative to the comparison group; #p<0.05 compared to pre-treatment parameters.*

the dermis was observed immediately after autofibroblast transplantation (by 16 %; p<0.05), after 6 and 12 months (by 23% and 27%, respectively; p<0.05).

Corneometry (epidermal moisture content) in Group 1 (25-35 years) before treatment did not differ from the GP level, increased after autofibroblast administration by 30 % (p<0.001) compared to the pre-treatment level, and remained elevated by 26 % (p<0.001) after 6 months. In the other three groups, they were lower than in GP: in Group 2-by 19 % (p<0.001), in Group 3 – by 30 % (p<0.001), in Group 4-by 40 % (p<0.001). Indicators increased relative to the pre-treatment level as a result of PRP administration [in Group 2 – by 17 % (p<0.001), 3rd-by 29 % (p<0.001), 4th – by 13 % (p<0.05)] and then remained at a high level (p<0.05) until the end of the examination.

Tevv indicators in patients with signs of physiological skin aging are shown in Table 2.

In women aged 25-35 years, the TEVV index before treatment did not differ from the GP level. In this group, as a result of neofibrolifting, it significantly decreased by 22 % (p<0.05) relative to the pre-treatment index 12 months after autofibroblast transplantation. In Group 2 (36-45 years), the tevv index before treatment also did not differ from the GP level. 6 months after fibroblast administration, it significantly decreased by 16% (p<0.05) compared to pre-treatment parameters and data after PRP administration and ablative fractional photothermolysis (p<0.05).

A 25% decrease in TEVV relative to pre-treatment parameters (p<0.01) was also observed after 12 months, but during this period the indicator became significantly lower (p<0.05) relative to GP (p<0.05). In Group 3, TEVV before treatment was 31% higher than in GP (p<0.001). The indicator significantly decreased relative to pre-treatment data: immediately after fibroblast administration by 17 % (p<0.05), 6 and 12 months after that by 28 % (p<0.05). In Group 4 (56 years and older), TEVV before treatment was 65% higher than in GP (p<0.001). A significant decrease in TEVV by 13 % (p<0.05) relative to the pre – treatment level was observed immediately after fibroblast administration, 22 % (p<0.01) – after 6, 19 % (p<0.05) - 12 months. At 6 and 12 months, the decrease in TEVV was also significant (p<0.05) relative to the level after PRP administration.

In the dynamics of neofibrolifting, there were significant changes in the OSC in the forehead area. In the younger group, OSH increased after fibroblast transplantation, and in the older group, this occurred after PRP administration and ablative fractional photothermolysis and persisted up to and including 12 months of follow-up. Almost similar changes in this indicator were observed in the cheek area. OSC in the cheek area in women aged 25-35 years before treatment did not differ from the level of GP. The rate increased significantly (by 28 %; p<0.01) compared to similar treatment immediately after autofibroblast transplantation and remained at a high level until the end of follow-up (p<0.01). During these periods, OSC in the cheek area was significantly higher (p<0.01) relative to the level of the indicator after PRP administration and ablative fractional photothermolysis and in the GP. OSHC in the cheek area in Group 2 before treatment was 35 % (p<0.01) lower than in GP, significantly increased relative to the pre-treatment level immediately after autofibroblast transplantation (80 %; p<0.001), and remained at a high level until the end of follow-up (p<0.001). During these

Table 2. Indicators of transepidermal moisture loss in patients of different age groups in the dynamics of treatment.

Age groups	Statistical indicators	TEVV indicators, G / H/M² In the comparison group yannya	Before treatment nya	During treatment after entering PRP and ablative fractional photothermolysis	Fibroblasts	After treatment via 6 months	12 months
1-ша	Me 25-75% n	11.9 9,.6-13.2 12	12.1 9.3-13.4 14	12.2 9.4-13.5 14	11.7 8.9-13.0 14	10.1 8.6-10.7 8	9.4* 7.9-10.0 8
2nd floor	Me 25-75% n	11.9 9.6-13.2 12	12.4 10.4-14.4 15	12.5 10.5-14.5 15	10.6 8.6-12.6 15	10.4# 9.1-10.6 7	9.3*# 8.0-9.5 7
3-тя	Me 25-75% n	11.9 9.6-13.2 12	15.6* 13.9-18.5 13	13.8* 12.1-16.7 13	13.0# 11.3-15.9 13	11.2# 10.6-15.3 9	11,.3# 10.7-15.4 9
4-та	Me 25-75% n	11.9 9.6-13.2 12	19.6* 17.2-21.9 12	18.8* 16.4-21.1 12	17.1*# 14.7-19.4 12	15.2*# 12.7-17.5 8	15.8*# 13.3-18,.1 8

*Note: *p<0.05 relative to the comparison group; #p<0.05 compared to pre-treatment parameters.*

periods, OSC in the cheek area was significantly higher ($p<0.001$) relative to the level of the indicator after PRP administration and ablative fractional photothermolysis and in the GP. After 12 months, OSC significantly decreased compared to the value after 6 months ($p<0.05$). OSC in the cheek area in the 3rd group of patients before treatment was 53% ($p<0.05$) less than in GP. The indicator increased relative to the pre – treatment level by 70 % ($p<0.001$) after PRP administration, 170 % ($p<0.001$) - fibroblasts, then the indicators remained at a high level ($p<0.001$) during the entire follow-up period. OSC in the cheek area in this group was significantly lower compared to GP after PRP administration and ablative fractional photothermolysis ($p<0.05$) and significantly more after autofibroblast transplantation ($p<0.01$), at 6 and 12 months ($p<0.05$). Almost similar data were obtained in Group 4, with the exception of a decrease in the OSHC index in the cheek area to the GP level 12 months after the introduction of autofibroblasts.

The results obtained indicate that neofibrolifting caused significant anti-aging structural and functional changes in the skin. The data determined the effective impact of PRP and ablative fractional photothermolysis on Aging Skin, which often manifests itself soon after its introduction. Also PRP and ablative fractional photothermolysis effectively prepare the skin for fibroblast autotransplantation, which is most likely due to pro-inflammatory cytokines and growth factors secreted by platelets and stimulated skin cells.

Taking into account modern ideas about the skin as a secondary organ of immunity, it could be assumed that one of the most adequate approaches to correcting involutional changes would be the prevention and elimination of immunosenescence.

As a result of neofibrolifting, the TEVV index in the younger group (25-35 years) significantly decreased relative to the pre-treatment index 12 months after autofibroblast transplantation by 22 % ($p<0.05$). In the 2nd group of patients, the tevv index before treatment also did not differ from the GP level. It significantly decreased 6 months after fibroblast administration by 16% ($p<0.05$) compared to the pre-treatment parameters obtained after PRP administration and ablative fractional photothermolysis ($p<0,05$).

A 25% reduction in TEVV ($p<0.01$) compared to pre-treatment values was also observed after 12 months. But during this period, the indicator also became significantly ($p<0.05$) lower relative to the GP ($p<0.05$). In Group 3, TEVV before treatment was 31 % ($p<0.001$) higher than in GP, significantly decreased (by 17 %; $p<0.05$) relative to the pre-treatment level immediately after fibroblast administration, 6 and 12 months after that (by 28 %; $p<0.05$). In Group 4, TEVV before treatment was 65 % ($p<0.001$) higher than in GP. There was a significant decrease in this indicator relative to the pre-treatment level immediately after fibroblast administration (by 13 %; $p<0.05$), after 6 (by 22 %; $p<0.01$) and 12 (by 19 %; $p<0.05$) months. At 6 and 12 months, the decrease in TEVV was also significant ($p<0.05$) compared to data after PRP administration and ablative fractional photothermolysis.

The content of CD3+cells in the culture of lymphocytes from skin biopsies of patients of Groups 1 and 2 did not significantly differ from the values of GP. In women of the 3rd and 4th groups, the CD3+cell content was significantly lower than the GP level (by 15% and 28%, respectively; $p<0.05$) and lower than the data of patients of the 2nd group ($p<0.05$ and $p<0.01$, respectively). The content of CD4+cells in women of Groups 1 and 2 did not differ from the GP values. In patients of the 3rd and 4th groups, the CD4+cell content was significantly lower than the GP level (by 12% and 25%, respectively; $p<0.05$) and lower than the data of women of the 2nd group ($p<0.05$ and $p<0.01$, respectively). In Group 4, the indicator was significantly lower ($p<0.05$) than in Group 1. The content of CD8+cells in the culture of lymphocytes from skin biopsies of patients in Group 1 did not differ from the values of GP. In women of the 2nd, 3rd and 4th groups, the CD8+cell content was significantly lower than the GP level (by 23%, 24% and 14%, respectively; $p<0.05$) and lower than the data of patients of the 1st group ($p<0.05$). But in the 4th group, the indicator was significantly higher ($p<0.05$) than in the 2nd. The content of CD19+cells in the culture of lymphocytes from skin biopsies of patients of the 1st and 2nd groups did not differ from the values in GP. In women of groups 3 and 4, the content of CD19+cells was significantly higher than the GP level [by 47 % ($p<0.01$) and 47 % ($p<0.001$), respectively] and higher than in patients of Group 2 ($p<0.01$).

In Group 1, the content of CD3+ -, CD4+ -, CD8+ -, and CD19+cells in women before treatment did not differ from the values in GP. The content of CD3+- and CD19+-cells during treatment in this group did not change significantly. After entering PRP and ablative fractional photothermolysis the number of CD4+lymphocytes increased by 29 % ($p<0.05$) relative to the pre-treatment level, and the number of CD8+ lymphocytes decreased by 19 % ($p<0.05$). After autofibroblast transplantation, the number of CD4+lymphocytes increased by 29 % ($p<0.05$) relative to the pre-treatment level, and CD8+lymphocytes decreased by 30 % ($p<0.05$).

In Group 2, the content of CD3+ -, CD4+- and CD19+- cells in the culture of lymphocytes from skin biopsies of

patients before treatment did not differ from the values of GP (Table 3).

The content of CD8+lymphocytes before treatment was 23% lower than that of GP ($p<0.05$). In this group after the introduction of PRP and ablative fractional photothermolysis the number of CD3+lymphocytes increased by 9 % ($p<0.05$) relative to the pre-treatment level, CD4+lymphocytes-by 16 % ($p<0.05$), the number of CD8+lymphocytes decreased by 15 % ($p<0.05$). After autofibroblast transplantation, the number of CD3+lymphocytes increased by 7 % ($p<0.05$) relative to the pre-treatment level, CD4+lymphocytes-by 16 % ($p<0.05$), and the number of CD8+ lymphocytes decreased by 25 % ($p<0.05$). The content of CD19+cells during treatment in this group did not change significantly.

In Group 3, the quantitative subpopulation composition in the culture of lymphocytes from skin biopsies of patients before treatment was changed: the content of CD3+lymphocytes was 15% lower than that of GP ($p<0.05$), CD4+lymphocytes-12 % ($p<0.05$), CD8+ lymphocytes-24 % ($p<0.05$), and CD19+lymphocytes – 47% higher ($p<0.01$).

In Group 4, the quantitative subpopulation composition in the culture of lymphocytes from skin biopsies of women before treatment was changed: the content of CD3+lymphocytes was 28% lower than that of GP ($p<0.05$), CD4+lymphocytes-25 % ($p<0.05$), CD8+ lymphocytes-14 % ($p<0.05$), and CD19+lymphocytes – 47% higher ($p<0.01$). In this group after the introduction of PRP and ablative fractional photothermolysis the number of CD8+lymphocytes decreased relative to the pre-treatment level by 14 % ($p<0.05$), CD19+lymphocytes-by 18 % ($p<0.001$). After autofibroblast transplantation, the number of CD3+lymphocytes was increased by 12 % ($p<0.05$) relative to the pre-treatment level, CD4+lymphocytes-by 32 % ($p<0.05$), CD8+ lymphocytes – by 13 % ($p<0.01$), CD19+lymphocytes - by 19 % ($p<0.01$). Prior to the results obtained, the data obtained in a long-term experiment were fundamentally similar.

The content of CD4+cells in the culture of lymphocytes from skin biopsies of patients of the 1st and 2nd groups did not differ from the values of GP. In women of groups 3 and 4, the indicator was significantly lower than the GP level [by 13 % ($p<0.05$) and 39 % ($p<0.001$)] and lower than the data of Group 2 ($p<0.05$ and $p<0.001$, respectively).

The content of CD8+cells in patients of Group 1 did not differ from the values of GP. In women of the 2nd, 3rd and 4th groups, the indicator was significantly lower than the GP level [by 29 % ($p<0.05$), 56 % ($p<0.001$), 27 % ($p<0.05$), respectively] and lower than the data of the 1st group ($p<0.01$; $p<0.001$ and $p<0.01$, respectively).

As a result of neofibrolifting, the content of CD4+- and CD8+-lymphocytes changed significantly, and in opposite directions. In Group 1, this indicator in patients before treatment did not differ from the values of GP (Table 4). After entering PRP and ablative fractional photothermolysis the number of CD4+lymphocytes increased by 19 % ($p<0.05$) relative to the pre-treatment level, while CD8+lymphocytes decreased by 24 % ($p<0.01$). After autofibroblast transplantation, the number of CD4+lymphocytes increased by 41 % ($p<0.001$) relative to the pre-treatment level, and CD8+lymphocytes decreased by 47 % ($p<0.001$). After 6 and 12 months, the number of CD4+lymphocytes increased by 84

Table 3. Content of lymphocytes of various subpopulations in the culture of lymphocytes from skin biopsies in patients of the 2nd age group (36-45 years) in the dynamics of treatment.

Subpopulations of lymphocytes	Statistical indicators	Content of lymphocytes in different subpopulations, %			
		Comparison group	Before treatment	During treatmentafter entering PRP and ablative fractional photothermolysis	Fibroblasts
CD3+	Me 25-75% n	61.5 50.5-72.8 10	68.7 48.6-70.8 17	74.7*# 54.6-76.8 17	73.5# 53.4-75.6 17
CD4+	Me 25-75% n	40.2 34.2-46.5 10	44.0 33.9-46.1 17	51.1*# 41.0-53.2 17	51.1*# 41.0-53.2 17
CD8+	Me 25-75% n	25.7 22.2-28.9 10	19.8* 18.9-25.9 17	16.8*# 15.9-22.9 17	14.9*# 14.0-21.0 17
CD19+	Me 25-75% n	10.8 8.5-13.0 10	13.7 9.5-14.3 17	12.3 8.1-12.9 17	12.9 8.7-13.5 17

*Note: *p<0.05 relative to the comparison group; #p<0.05 compared to pre-treatment parameters.*

% (p<0.001) and 36 % (p<0.05), respectively, relative to pre-treatment levels, and CD8+lymphocytes decreased by 60 % (p<0.001) and 43 % (p<0.01), respectively.

In the 2nd group of patients, the content of CD4+cells before treatment did not differ from the values of GP. The CD8+cell content was 29% lower than the GP level (p<0.01). After entering PRP and ablative fractional photothermolysis the number of CD4+lymphocytes increased by 31 % (p<0.01) relative to the pre-treatment level, while CD8+lymphocytes decreased by 37 % (p<0.01). After autofibroblast transplantation, the number of CD4+lymphocytes increased by 51 % (p<0.001) compared to the pre-treatment level, and CD8+lymphocytes decreased by 44 % (p<0.001). After 6 and 12 months, the number of CD4+lymphocytes relative to the pre-treatment level was increased by 76 % (p<0.001) and 80 % (p<0.001), respectively, and CD8+ lymphocytes decreased by 53 % (p<0.001) and 49 % (p<0.001), respectively. In patients of the 3rd Age Group, an increase in the number of CD4+lymphocytes in the skin occurred after PRP administration and ablative fractional photothermolysis and rose to a higher level as a result of autofibroblast transplantation, persisting until the end of follow-up. The number of CD8+ lymphocytes was significantly reduced before treatment and further decreased after autofibroblast transplantation only after 6 months, and after 12 months the indicator returned to the value that it had before treatment.

In Group 3, the content of CD4+cells before treatment was 13% lower than the GP level (p<0.05), CD8+cells-56 % (p<0.05). The number of CD4+lymphocytes relative to the pre-treatment level increased by 43% (p<0.05) after PRP administration and ablative fractional photothermolysis, 70% (p<0.05) – autofibroblast transplants. After 6 and 12 months, CD4+lymphocyte counts were increased relative to pre-treatment levels by 160 % (p<0.001) and 120 % (p<0.001), respectively. The number of CD8+lymphocytes changed significantly only after 6 months and was 49% lower than before treatment (p<0.05).

In the 4th group of patients, the content of CD4+cells before treatment was 39% lower than the GP index (p<0.001) (Table 5), CD8+cells-27 % (p<0.05). After entering PRP and

Table 4. CD4 content+- and CD8+- lymphocytes in the culture of lymphocytes from skin biopsies in patients of the 1st age group (25-35 years) in the dynamics of treatment.

Ulation SubPop lymphoc ИТІВ	Statistics show ИКИ	Comparison on group	Content of lymphocytes in different subpopulations, % Before treatment	During treatment after entering PRP and ablative fractional photothermolysis	Fibroblasts	After treatment via 6 months	12 months
CD4+	Me	29.1	31.7	37.8*#	44.6*#	58.3*#	43.3*#
	25-75 %	23.3-34.4	22.5-33.9	28.6-40.0	35.4-46.8	55.2-60.1	40.1-45.0
	n	12	14	14	14	8	8
CD8+	Me	25.6	24.3	18.4*#	12.9*#	9.7*#	13.9*#
	25-75 %	22.2-28.9	22.7-30.2	16.8-24.3	11.3-18.8	9.2-12.0	13.4-16,.1
	n	12	14	14	14	8	8

*Note: *p<0.05 relative to the comparison group; #p<0.05 compared to pre-treatment parameters.*

Table 5. CD4 content+- and CD8+- lymphocytes in the culture of lymphocytes from skin biopsies in patients of the 4th age group (56 years and older) in the dynamics of treatment.

Pool subpoena lymphoc ИТІВ	Statisticians of chni indicator and	Group comparison	Content of lymphocytes in different subpopulations, % Before treatment	During treatment after entering PRP and ablative fractional photothermolysis	Fibroblasts	After treatment via 6 months	12 months
CD4+	Me	29.1	17.7*	35.9*#	44.4*#	42.0*#	44.7*#
	25-75 %	23.3-34.4	13.9-22.3	30.0-42.7	38.5-51.2	40.2-54.1	42.9-56.8
	n	12	12	12	12	8	8
CD8+	Me	25.6	18.8*	11.5*#	12.5*#	11.5*#	13.9*#
	25-75 %	22.2-28,.9	15.0-23.3	7.7-16.1	8.7-17.1	11.0-12.8	8.0-15.5
	n	12	12	12	12	8	8

*Note: * 0.05 relative to the comparison group; #p<0.05 compared to pre-treatment parameters.*

ablative fractional photothermolysis the number of CD4+lymphocytes relative to the level before treatment increased by 101 % ($p<0.001$), CD8+lymphocytes-decreased by 39 % ($p<0.01$). After autofibroblast transplantation, the number of CD4+lymphocytes relative to the level before treatment was increased by 151 % ($p<0.001$), CD8+lymphocytes-decreased by 34 % ($p<0.05$). After 6 and 12 months, the number of CD4+lymphocytes relative to the pre-treatment level was increased by 138 % ($p<0.001$) and 153 % ($p<0.001$), CD8+lymphocytes-decreased by 39 % ($p<0.001$) and 26 % ($p<0.05$), respectively.

Thus, as a result of the study of the participation of lymphocytes in neofibrolifting, it was found that the clinical effect correlated with changes in the number of CD3+ -, CD4+ -, CD8+-subpopulations of T-lymphocytes and CD19+-B-cells in the skin. This indicates their likely involvement in the implementation of the effect of the method as a result of both PRP administration and fibroblast autotransplantation. Important for assessing the prospects of the procedure is the established duration of immune changes.

Conclusions

Involutional changes in the skin of women of different ages are characterized by a significant decrease in acoustic density (by 22% in the comparison group), thickness of the epidermis (by 22% in the 3rd Group, 21%-4th) and dermis (by 21% in the 2nd Group, 10%-3rd, 24% – 4th), volume velocity of blood circulation in the forehead (by 22% in the 3rd Group, 72%-4th) and cheeks (by 35% in the 2nd Group, 53%-3rd, 69% – 4th), skin hydration (by 19% in Group 2, 30%-3rd, 40% – 4th) together with increased transepidermal water loss (by 31% in Group 3, 65%-4th).

It was determined that with age, the number of CD3+-T-lymphocytes in the skin significantly decreases relative to the comparison group indicators (by 15% in Group 3, 28%-4), CD4+ – T-lymphocytes (by 12% in Group 3, 25%-4), CD8+-T-lymphocytes (by 23% in Group 2, 24% – 3, 14%-4) and the number of CD19+-B-cells increases (by 47% in Group 3-th and 4th groups).

3. To correct involutional-dystrophic skin changes, the neofibrolifting technique has been improved, including preparatory injections of platelet-rich plasma, ablation fractional photothermolysis procedure and after 2 weeks – transplantation of cultured autologous dermal fibroblasts into conditioned skin areas.

It was found that as a result of neofibrolifting, the acoustic density of the skin significantly increased relative to the indicators before treatment in patients of the 1st and 2nd groups after 12 months by 19% and 33%, respectively; the 3rd and 4th groups after 6 months by 17% and 31%, 12 months by 25% and 34%, respectively. The thickness of the epidermis in women of Group 2 after dermal fibroblast transplantation increased by 17 %; in patients of Group 3 after platelet-rich plasma administration and ablative fractional photothermolysis – 20 %, dermal fibroblast transplants – 22%, after 6 and 12 months-22% and 23%, respectively; in people of the 4th group after dermal fibroblast transplantation – 19%, after 6 months – 24%. Dermal thickness in Group 1 patients after platelet-rich plasma administration and ablative fractional photothermolysis increased by 9 %, dermal fibroblast transplants – 11 %, after 6 months – 18 %; in women of the 2nd and 3rd groups-28% and 19%, respectively; in people of the 4th group after dermal fibroblast transplantation-16%, after 6 and 12 months – 23% and 27%, respectively.

Skin hydration in Group 1 patients after dermal fibroblast transplantation increased by 30%, after 6 months – by 26 %; in Group 2 women after platelet-rich plasma administration and ablative fractional photothermolysis – 17 %, dermal fibroblast transplants-30% , after 6 and 12 months – 35% and 31%, respectively; in individuals of the 3rd Group after administration of platelet-rich plasma and ablative fractional photothermolysis – 29 %, dermal fibroblast transplants-37%, after 6 and 12 months – 28% and 29%, respectively; in patients of Group 4 after platelet-rich plasma administration and ablative fractional photothermolysis – 13 %, dermal fibroblast transplants – 32%, after 6 and 12 months-29% and 18%, respectively. Transepidermal moisture loss in women of Group 1 after 12 months decreased by 22 %; in patients of Group 2 after 6 and 12 months-16% and 25%, respectively; in patients of Group 3 after dermal fibroblast transplantation-17%, after 6 and 12 months – 28% and 28%, respectively; in patients of Group 4 after dermal fibroblast transplantation-13%, after 6 and 12 months – 22% and 19%, respectively.

It was found that as a result of neofibrolifting, blood flow parameters were normalized. The volume rate of blood flow in the forehead area in women of Group 1 after dermal fibroblast transplantation increased by 29 %, at 6 and 12 months-34% and 49%, respectively; in patients of Group 2 after platelet – rich plasma administration-24%, dermal fibroblast transplantation-24%, at 6 and 12 months – 78% and 92%, respectively; in persons of Group 3 after platelet-rich plasma administration and ablative fractional photothermolysis – 50 %, dermal fibroblast transplants-79% , after 6 and 12 months – 88% and 69%, respectively; in patients of the

4th group after administration of plateletrich plasma and ablative fractional photothermolysis – 3.5 times, dermal fibroblast transplants – 4 times, after 6 and 12 months-4.1 and 4 times, respectively. The volume rate of blood flow in the shock area in women of Group 1 after dermal fibroblast transplantation increased by 28 %, at 6 and 12 months-34% and 40%, respectively; in patients of Group 2 after dermal fibroblast transplantation – 1.8 times, at 6 and 12 months-2.1 and 2 times, respectively; in persons of Group 3 after platelet-rich plasma administration and ablative fractional photothermolysis – 1.7 times, dermal fibroblast transplants-2.7 times, after 6 and 12 months – 2.8 and 2.7 in patients of the 4th group after administration of platelet-rich plasma – 1.9 times, dermal fibroblast transplantation – 4 times, after 6 and 12 months – 4.1 and 3.6 times, respectively.

It was found that as a result of neofibrolifting, an increase in the number of CD4+cells occurred, as well as a decrease in the number of CD8+lymphocytes with the restoration of the ratio of the number of these cells to the normal level of young people. Number of CD4 + cells in Group 1 patients after platelet-rich plasma administration it increased by 19%, after dermal fibroblast transplantation – 41%, after 6 and 12 months-84% and 36%, respectively; in women of Group 2 after platelet-rich plasma administration and ablative fractional photothermolysis - 31 %, dermal fibroblast transplants-51% , after 6 and 12 months – 76% and 80%, respectively; in individuals of the 3rd Group after administration of platelet-rich plasma and ablative fractional photothermolysis – 1.4 times, dermal fibroblast transplants - 1.7 times, after 6 and 12 months – 2.6 and 2.2 times, respectively; in patients of the 4th group after administration of platelet-rich plasma and ablative fractional photothermolysis – 2 times, dermal fibroblast transplants – 2.5 times, after 6 and 12 months-2.4 and 2.5 times, respectively. CD8+cell count in Group 1 women after platelet-rich plasma administration and ablative fractional photothermolysis decreased by 24 %, dermal fibroblast transplants-47%, after 6 and 12 months-60% and 43%, respectively; in patients of Group 2 after platelet-rich plasma administration and ablative fractional photothermolysis – 37 %, dermal fibroblast transplants – 44%, after 6 and 12 months-53% and 49%, respectively; in people of the 3rd Group after 6 months – 49%; in patients of the 4th group after administration of plateletrich plasma-39%, dermal fibroblast transplants – 34%, after 6 and 12 months – 39% and 26%, respectively.

Positive clinical, structural-functional and immunological data indicating the anti-aging effect of neofibrolifting are often significant after administration of platelet-rich plasma and ablative fractional photothermolysis and as a result of subsequent transplantation of dermal fibroblasts with the formation of a stable reliable comprehensive result, which is available during the entire 12-month follow-up period. On the one hand, this indicates a significant role in the realization of the immuno – inflammatory effect of platelet-rich plasma, on the other hand, it highlights the main and long-term effect of activated transplanted dermal fibroblasts.

Availability of data and materials: All data generated or analyzed during this study are included in this published article.

Ethics approval and consent to participate: The Ethics Committee of Shupyk National Healthcare University of Ukraine approved this study). The study is conformed with the Helsinki Declaration of 1964, as revised in 2013, concerning human and animal rights. All patients participating in this study signed a written informed consent form for participating in this study.

Informed consent: Written informed consent was obtained from a legally authorized representative(s) for anonymized patient information to be published in this article.

Conflict of interest: The Authors declare no conflict of interest.

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