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Original article

Application of autologous fibroblasts in correcting involution-dystrophic skin changes

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Abstract. This article delves into the clinical, instrumental and immunological studies of facial involutional-dystrophic skin changes in female patients of different ages with the development of a clinical method within their treatment called neofibrolifting. This method utilizes the transplant of autologous fibroblasts into the pretreated patient with platelet-rich plasma skin. Planning the proper treatment plan was based on the data of the transplanted cells properties as well as the instrumental and immunological studies of the patients' dermis. Aging is a complex biological process of metabolic and structural-functional changes in the body, covering all human organs and tissues. Skin aging is part of the irreversible biological processes occurring in the body and is caused by genetic disorders, telomere shortening, resistance of cellular structures to oxidative damage, as well as aggressive environmental influences[1]. The properties and functions of the skin and its appendages deteriorate with age, the skin loses moisture, the ability to regenerate, becomes thinner, and the processes of keratinization, pigmentation, blood circulation, collagen synthesis, etc.are disrupted[2,3]. There is an accumulation of data on the significant involvement of the involution of the immune system in overall skin aging, which is generally defined as the process of immunosenescence[4,5]. This manifests through mechanisms known as age-related disorders in the compartments of hematopoietic stem and multipotent stromal cells with the inhibition of hematopoiesis and disorders in the regulation of immunoneuroendocrine[6,7]. There is a shift of cellular cooperative mechanisms towards the formation of chronic inflammations with the increased production of pro-inflammatory cytokines by adaptive and innate immune cells, which cause the appearance of a large number of senescent and terminally differentiated cells that are not able to perform the necessary functions[8]. Immunosenescent atrophic and dystrophic phenomena in the skin are manifested by a pronounced decrease in the number of dermal fibroblasts with the inhibition of their functional activity, which also negatively affects the condition of keratinocytes. As a result, the normal process of renewal of dermal fibroblasts and keratinocytes, the formation of the intercellular matrix, is disrupted, which primarily causes the appearance of noticeable involutional signs[9-11].

Key words: fibroblasts, platelet rich plasma, aiging, skin

The research objective

Optimization of correcting involutional-skin changes in women with physiological aging by applying autologous fibroblasts, taking into account age-related structural and functional, microcirculatory and immunological features of the skin.

Materials and methods

This work presents the data of a survey of 107 patients with signs of age-related facial skin changes ages 25 to 60 years, which was conducted on the clinical basis of the Department of dermatovenerology of the P. L. Shupik National Medical Academy

of postgraduate education (Institute of plastic surgery Virtus).

The criteria for inclusion in the study were: the presence of signs of involutional skin changes, the female gender, age ranging from 25 to 60 years, a signed informed consent to inclusion in the study; exclusion criteria – skin diseases in the active phase, herpetic and other infectious processes on the skin during the acute period, general infectious diseases, chronic somatic diseases in the acute stage, mental diseases, epilepsy, a tendency to form keloid scars, oncological diseases, pregnancy, lactation, pathology of the blood coagulation system, severe immunosuppression, connective tissue diseases (Ehlers syndrome-Danlos, scleroderma).

All patients were divided into groups based on age: 1st - 25-35 years (n=22), 2nd - 36-45 (n=32), 3rd - 46-55 (n=28), 4th – 56 and older (n=22).

The comparison group (CG) consisted of 22 women aged 25-35 years without visual signs of Chrono - and photoaging, which were studied to be immunological and structural-functional indicators of the skin.

The study of the structural and functional state of the skin was executed through the use of instrumental methods. To assess structural changes in the skin, ultrasound dermascanning was used by means of a portable high – frequency ultrasound device "DUB - Digital Ultraschall Bildsystem-tpm" and DUB-SkinScan ver software.3.2 (Germany). The moisture content in the epidermis was estimated by corneometry, based on measuring the electrical capacitance of a dielectric medium. The state of the barrier function of the epidermis was studied by measuring TEWL (transepidermal water loss). For these studies, a multi Skin Test center® MC 1000 diagnostic workstation (Courage+Khazaka electronic GmbH, Germany) was used. Blood flow measurements were performed using an ultrasound Doppler scanning using the Minimax-Doppler-K device (Russia). VBF was measured in the skin of the forehead and cheeks (ml /s/ cm^3).

Skin sampling for the cultivation and production of fibroblasts, as well as immunological studies, was performed using a biopsy method. Tissue samples were placed in a sterile container and transferred to the biotechnological laboratory equipped according to the requirements of the 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 antibody kits for cell phenotyping BD Multitest IMK Kit (BD Multitest™ CD3FITC/CD8PE/CD45PerCP/CD4APC reagent, BD Multitest CD3FITC/CD16PE+CD56PE/CD45PerCP/CD19APC reagent) in compliance with the manufacturer's recommendations for use of equipment and reagents.

The harvest Smart PReP2 automatic centrifuge (USA) was used to produce platelet-rich plasma (PRP).

Protocol for the study and treatment of patients are shown in Figure 1.

After clinical, laboratory and instrumental examinations, 107 patients of different ages underwent skin biopsy to culture dermal fibroblasts and study the immunological parameters. After 2 weeks, the administration of PRP was performed in the form of intradermal injections (14 ml), after another 2 – skin biopsy for immunological studies and autofibroblast transplantation in the form of intradermal injections (60 million cells), instrumental studies. Another 2 weeks after the fibroblast transplant, a skin biopsy was performed for immunological studies, and instrumental studies were performed.

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

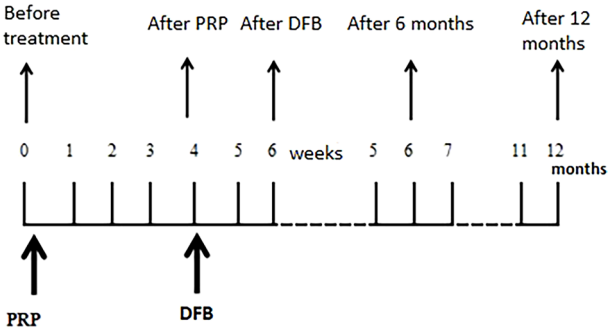

Figure 1. Treatment regimen and patient studies.

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

Discussion and conclusions

In the 1st group of women (25-35 years old) early age-related changes were identified, 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, and acquired ptosis of the 3rd degree (grades 3B, 3B) were noted.

The thickness of the epidermis in patients of the 1st and 2nd groups did not significantly differ from the indicators of practically healthy individuals, and in patients 46 years and older there was a significant decrease in the thickness of the epidermis in relation to CG: in the 3rd group - by 22 % ($p<0.05$), the 4th - by 21 % ($p<0.05$). The thickness of the dermis in women ages 25-35 years was almost the same as that of CG. In older age groups, the indicator significantly decreased in relation to CG (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 CG. In the 2nd and 3rd groups, an unreliable decrease in this parameter was identified. Only group 4 showed a 22% decrease in skin acoustic density ($p<0.05$) compared to CG and Group 1.

In Group 1, the results of corneometry did not differ noticeably 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 CG 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 ($p<0.001$). Progressively 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, TEWL did not significantly increase relative to CG 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 TEWL index significantly increased relative to the CG 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 ($p<0.01$) significantly 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 (Sandy-Moller J. et al., 2003).

VBF (volumetric blood flow) in the forehead area in the 2nd group of patients decreased relative to the level of CG, although irregularly. In Group 3, there was a significant decrease in the indicator by 22 % ($p<0.05$) relative to the CG. In the oldest age group, VBF in the forehead area decreased by 72% relative to the CG ($p<0.05$) and relative to other groups ($p<0.001$). When studying VBF in the cheek area, it turned out that it significantly decreased by 35 % ($p<0.01$) in the 2nd group compared to the CG and 1st group ($p<0.01$). In future analyses, there was an even greater decrease in VBF in the cheek area relative to the CG (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 appropriate to induce an inflammatory process in the skin before the introduction of fibroblasts by using preliminary platelet injections, which ensure the appearance of pro-inflammatory cytokines and growth factors in the tissue, which significantly activate the proliferation and differentiation of the fibroblasts.

Under the influence of neofibrolifting, significant changes in data indicators occurred. However, the thickness of the epidermis in Group 1 (25-35 years) did not differ from the value in CG and did not change during neofibrolifting (Table 1). In Group 2 (36-45 years), it did not differ from CG 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 the CG ($p<0.05$). In this group, a 20% increase in this indicator ($p<0.05$) relative to pre-treatment data occurred after PRP management and was at the same level as a result of autofibroblast administration 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 CG ($p<0.05$). Immediately after the autofibroblast transplantation, the thickness of the epidermis also increased (by 19 %; $p<0.05$), but remained at the achieved level only for a period of 6 months. The introduction of PRP in this group was ineffective.

The thickness of the dermis in the youngest group of patients (25-35 years) before treatment was not differed from the CG level and increased by 9% relative to the pre – treatment index ($p<0.05$) after PRP administration, 11% ($p<0.05$) after fibroblast transplantation, and 18% ($p<0.05$) after a 6 months follow – up period. 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 CG, 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% ($p<0.05$).

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

Age groups	Statistical indicators	Epidermis thickness, microns					
		in the CG	before treatment	In the course of treatment after administration		time passed after treatment	
				PRP	fibroblasts	6 months.	12 months.
	Me	108.2	87.1	87,5	102,2#	98,1	95,6
2-nd	25-75 %	88,4-124.0	76,5-112,0	76,9-112,4	91,6-127,1	92.7-101,6	90,2-99,1
	n	12	15	15	15	7	7
	Me	108.2	84,7*	102,0#	103,2#	103,6#	103,9#
3-rd	25-75 %	88,4-124.0	63,4-94,3	80,7-111,6	81,9-112,8	84.7-112,3	85,0-112,6
	n	12	13	13	13	9	9
	Me	108.2	85,5*	83,4*	102,0#	105,6#	85,4*
4-th	25-75 %	88,4-124.0	73,4-102,1	71,3-100,0	89,9-118,6	97,4-117,1	77,2-96,9
	n	12	12	12	12	8	8

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

In patients 56 years and older, the thickness of the dermis before treatment was 24% less than that of CG ($p<0.05$). The response to the treatment in the fourth Group was most pronounced: the thickening of 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 CG 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 the CG: 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 level before treatment already 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.

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

In women ages 25-35 years, the TEWL index before the treatment did not differ from the CG 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 TEWL index before treatment also did not differ from the CG level. 6 months after fibroblast administration, it significantly decreased by 16% ($p<0.05$) compared to pre-treatment parameters and data after PRP administration ($p<0.05$).

A 25% decrease in TEWL 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 the CG ($p<0.05$). In Group 3, TEWL before treatment was 31% higher than in the CG ($p<0.001$). The indicator significantly decreased relative to pre-treatment data: immediately after the administration of fibroblasts

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

Age groups	Statistical indicators	TEWL indicators, gm/hr/m^2					
		in the comparison group	before treatment	In the course of treatment after administration		Time passed after treatment	
				PRP	fibroblasts	6 months.	12 months.
	Me	11.9	12,1	12,2	11,7	10,1	9,4*
1-st	25-75 %	9,6-13,2	9,3-13,4	9,4-13,5	8,9-13,0	8,6-10,7	7,9-10,0
	n	12	14	14	14	8	8
	Me	11.9	12,4	12,5	10,6	10,4#	9,3*#
2-nd	25-75 %	9,6-13,2	10,4-14,4	10,5-14.5	8,6-12,6	9,1-10,6	8,0-9,5
	N	12	15	15	15	7	7
	Me	11.9	15,6*	13,8*	13,0#	11,2#	11,3#
3-rd	25-75 %	9,6-13,2	13,9-18.5	12,1-16,7	11,3-15,9	10,6-15,3	10.7-15,4
	n	12	13	13	13	9	9
	Me	11.9	19,6*	18,8*	17,1*#	15,2*#	15,8*#
4-th	25-75 %	9,6-13,2	17,2-21,9	16,4-21,1	14,7-19,4	12,7-17,5	13,3-18,1
	n	12	12	12	12	8	8

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

by 17% ($p<0.05$), 6 and 12 months after that by 28% ($p<0.05$). In Group 4 (56 years and older), TEWL before treatment was 65% higher than in CG ($p<0.001$). A significant decrease in TEWL by 13% ($p<0.05$) relatively 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 TEWL was also significant ($p<0.05$) relative to level after PRP administration.

In the dynamics of neofibrolifting, there were significant changes in the VBF in the forehead area. In the younger group, VBF increased after fibroblast transplantation, and in the older group, this occurred after PRP administration and persisted up to and including a 12 months follow up period. Almost similar changes in this indicator were observed in the cheek area. VBF in the cheek area in women aged 25-35 years before treatment did not differ from the level of the CG. The rate increased significantly (by 28%; $p<0.01$) compared to the same treatment immediately after the autofibroblast transplantation and remained at a high level until the end of the follow-up ($p<0.01$). During these periods, VBF in the cheek area was significantly higher ($p<0.01$) relative to the level of the indicator after PRP administration and in CG. VBF in the cheek area in Group 2 before treatment was 35% ($p<0.01$) lower than in CG, 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 periods, VBF in the cheek area was significantly higher ($p<0.001$) compared to the level of the index after PRP administration and in the CG. After 12 months, VBF significantly decreased compared to the value it reached after 6 months ($p<0.05$). VBF in the cheek area in the 3rd group of patients before treatment was 53% ($p<0.05$) less than in the CG. 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. The VBF in the cheek area in this group was significantly lower compared to the CG after PRP administration ($p<0.05$) and significantly higher after autofibroblast transplantation ($p<0.01$), at 6 and 12 months ($p<0.05$). Similar data was obtained in Group 4, with the exception of a decrease in the OSHC index in the cheek area to the level of the CG 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 effect of PRP on Aging Skin, which often manifests itself soon after its administration. PRP also effectively prepares 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 TEWL 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 TEWL index before treatment also did not differ from the CG level. It significantly decreased 6 months after the adbiminstration of fibroblasts by 16% ($p<0.05$) compared to the pre-treatment parameters given after PRP administration ($p<0.05$).

A 25% reduction in TEWL ($p<0.01$) compared to pre-treatment values was also observed after 12 months. But during this period, the indicator also became significantly lower ($p<0.05$) relative to the CG ($p<0.05$). In Group 3, TEWL before treatment was 31% ($p<0.001$) higher than in the CG, significantly reduced (by 17%; $p<0.05$) relatively to the level before treatment immediately after fibroblast administration, and after 6 and 12 months (by 28 %; $p<0.05$). In Group 4, TEWL before treatment was 65% ($p<0.001$) higher than in the CG. 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 TEWL was also significant ($p<0.05$) compared to the data after the administration of PRP.

CD3 content^{+}-cells in the culture of lymphocytes from skin biopsies of patients of the 1st and 2nd groups did not significantly differ from the values of

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

In the 1st Group, the content of $CD3^+$-, $CD4^+$-, $CD8^+$- and $CD19^+$- cells in women before treatment did not differ from the values in CG. CD3 $content^+$- and $CD19^+$- the number of cells during treatment in this group did not change significantly. After PRP administration the amount of $CD4^+$- lymphocyte counts increased by 29% ($p<0.05$) relative to pre-treatment levels, CD8 $counts^+$- lymphocytes decreased by 19% ($p<0.05$). CD4 count after autofibroblast $transplantation^+$- lymphocytes were increased by 29% ($p<0.05$) relative to pre-treatment levels, $CD8^+$-lymphocytes-reduced by 30% ($p<0.05$).

In the 2nd Group, 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 CG (Table 3).

CD8 $content^+$- lymphocytes before treatment were 23% less than in the CG ($p<0.05$). In this group, after PRP administration, the amount of $CD3^+$- lymphocytes increased by 9% ($p<0.05$) relative to pre-treatment levels, $CD4^+$- lymphocytes - 16% ($p<0.05$), CD8 $count^+$- lymphocytes decreased by 15% ($p<0.05$). CD3 count after autofibroblast $transplantation^+$-lymphocytes were increased by 7% ($p<0.05$) relative to pre-treatment CD4 $levels^+$- lymphocytes - 16% ($p<0.05$), CD8 $count^+$- lymphocytes are reduced by 25% ($p<0.05$). CD19 $content^+$- the number of 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: CD3 $content^+$- lymphocytes were 15% less than in CG ($p<0.05$), $CD4^+$- lymphocytes - 12% ($p<0.05$), $CD8^+$- lymphocytes – 24% ($p<0.05$), and $CD19^+$-lymphocytes-47% more ($p<0.01$).

In Group 4, the quantitative subpopulation composition in the culture of lymphocytes from skin biopsies of women before treatment was changed: CD3 $content^+$- lymphocytes were 28% smaller than CG ($p<0.05$), $CD4^+$- lymphocytes-25% ($p<0.05$), $CD8^+$- lymphocytes – 14% ($p<0.05$), and $CD19^+$-lymphocytes-47% more ($p<0.01$). In this group, after PRP administration, the number of $CD8S^+$- lymphocytes decreased relative to the pre-treatment level by 14% ($p<0.05$), $CD19^+$- lymphocytes-18% ($p<0.001$). CD3 abundance after autofibroblast $transplantation^+$-lymphocytes were increased by 12% ($p<0.05$) relative to pre-treatment CD4 $levels^+$- lymphocytes-32% ($p<0.05$), $CD8^+$- lymphocytes-reduced by 13% ($p<0.01$), $CD19^+$- lymphocytes-19% ($p<0.01$).

Prior to the results obtained, the data obtained in a long-term experiment were fundamentally similar.

CD4 $content^+$-cells in the culture of lymphocytes from skin biopsies of patients of the 1st and 2nd groups did not differ from the values of the CG. In women of groups 3 and 4, the indicator was significantly lower than the CG 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).

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

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	In the course of treatment after administration	
				PRP	fibroblasts
$CD3^+$	Me	61,5	68,7	74,7*#	73,5#
	25-75 %	50,5-72,8	48,6-70,8	54,6-76,8	53,4-75,6
	n	10	17	17	17
Subpopulations of lymphocytes	**Statistical indicators**	**Content of lymphocytes in different subpopulations, %**			
		comparison group	**before treatment**	**Course of treatment after administration**	
				PRP	**fibroblasts**
$CD4^+$	Me	40.2	44.0	51,1*#	51,1*#
	25-75 %	34,2-46,5	33,9-46,1	41,0-53,2	41,0-53,2
	n	10	17	17	17
$CD8^+$	Me	25.7	19,8*	16,8*#	14,9*#
	25-75 %	22,2-28,9	18,9-25,9	15,9-22,9	14,0-21,0
	n	10	17	17	17
$CD19^+$	Me	10,8	13,7	12,3	12,9
	25-75 %	8,5-13,0	9,5-14,3	8,1-12,9	8,7-13,5
	n	10	17	17	17

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

the data of the 1st group (p<0.01; p<0.001 and p<0.01, respectively).

As a result of neofibrolift CD4 content$^+$- and $CD8^+$- the lymphocytes changed significantly, and in opposite directions. In Group 1, this indicator in patients before treatment did not differ from the values of CG (Table 4). After PRP administration the amount of $CD4^+$- lymphocytes increased by 19% (p<0.05) relative to pre-treatment levels, $CD8^+$- lymphocytes decreased by 24% (p<0.01). CD4 abundance after autofibroblast transplantation$^+$- lymphocytes were increased by 41% (p<0.001) relative to pre-treatment levels, $CD8^+$- lymphocytes-reduced by 47% (p<0.001). After 6 and 12 months the number of $CD4s^+$- lymphocytes were increased by 84% (p<0.001) and 36% (p<0.05), respectively, relative to pre-treatment levels, $CD8^+$- lymphocytes-reduced 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 CG values. CD8 content$^+$- cells were less than the CG level by 29% (p<0.01). After PRP administration the amount of $CD4^+$- lymphocytes increased by 31% (p<0.01) relative to pre-treatment levels, $CD8^+$- lymphocytes decreased by 37% (p<0.01). CD4 count after autofibroblast transplantation$^+$- lymphocytes were increased by 51% (p<0.001) relative to pre-treatment CD8 levels$^+$- lymphocytes-reduced by 44% (p<0.001). After 6 and 12 months CD4 abundance$^+$- lymphocytes relative to pre-treatment levels were increased by 76 % (p<0.001) and 80% (p<0.001), respectively, $CD8^+$- lymphocytes-reduced by 53% (p<0.001) and 49% (p<0.001), respectively. In patients of the 3rd age group, the number of CD4 increases$^+$- lymphocytes in the skin occurred after administration of PRP and increased to a higher level as a result of autofibroblast transplantation, persisting until the end of follow-up. CD8 numbers$^+$- lymphocytes were significantly reduced already before treatment and further decreased after autofibroblast transplantation only after

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.

Subpopulations of lymphocytes	Statistics indicators	Content of lymphocytes in different subpopulations, %					
		comparison group	before treatment	In the course of treatment after administration		after treatment	
				PRP	fibroblasts	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.

6 months, and in the 12-month period the indicator returned to the value that it had before treatment.

In Group 3, CD4 content$^+$- cells before treatment were 13% lower than the CG level (p<0.05), CD8$^+$– cells-56% (p<0.05). CD4 quantity$^+$- lymphocytes relative to the level before treatment increased by 43% (p<0.05) after PRP administration, 70% (p<0.05) – autofibroblast transplantation. After 6 and 12 months CD4 content$^+$- lymphocytes increased relative to pre-treatment levels by 160% (p<0.001) and 120% (p<0.001), respectively. CD8 quantity$^+$- lymphocytes changed significantly only after 6 months and were lower than the pre-treatment level by 49% (p<0.05).

In the 4th group of patients, the amount of CD4$^+$- cells before treatment were 39% less than CG (p<0.001) (Table 5), CD8$^+$– cells-27% (p<0.05). After PRP administration the amount of CD4$^+$- lymphocytes relative to the level before treatment increased by 101% (p<0.001), CD8$^+$- lymphocytes-decreased by 39% (p<0.01). CD4 abundance after autofibroblast transplantation$^+$- lymphocyte levels prior to treatment were increased by 151% (p<0.001), CD8$^+$-lymphocytes-reduced by 34% (p<0.05). After 6 and 12 months the number of CD4s$^+$- lymphocytes relative to pre-treatment levels were increased by 138% (p<0.001) and 153% (p<0.001), CD8$^+$-lymphocytes-reduced by 39% (p<0.001) and 26% (p<0.05), respectively.

Thus, as a result of the study of the use of lymphocytes in neofibrolifting, it was found that the clinical effect correlates with changes in the amount of CD3$^+$-, CD4$^+$-, CD8$^+$- subpopulations of T-lymphocytes and CD19$^+$- B-cells in the skin. This indicates their possible involvement in the implementation of the effect of the method as a result of both PRP administration and the autotransplantation of fibroblasts. To adequately rate the prospects of the procedure the established duration of immune changes are evaluated.

Conclusions

The paper presents theoretical generalization of structural - functional, immunological mechanisms and their relationship in the development of involutional facial skin changes in patients from different age groups and the solution of the scientific problem of improving the effectiveness of treatment by creating a new complex technique of neofibrolifting with autotransplantation of dermal fibroblasts into the skin conditioned with platelet-rich plasma.

1. It was found that involutional skin changes in women of different ages are characterized by a significant decrease in acoustic density (by 22% in the comparison group), thickness

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.

Subpopulations of lymphocytes	Statististical indicators	Content of lymphocytes in different subpopulations, %					
		group comparison	before treatment	In the course of treatment after administration		Time passed after treatment	
				PRP	fibroblasts	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: – p<0.05 relative to the comparison group; #p<0.05 compared to pre-treatment parameters.

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 skin hydration (by 19% in Group 2,30% in Group 3,40% in Group 4) together with an increased transepidermal water loss (by 31% in Group 3,65% in Group 4).

2. It was determined that with age, the amount of CD3 in the skin significantly decreases relative to the indicators of the comparison group$^+$-T-lymphocytes (by 15% in the 3rd Group, 28% in the 4th), CD4$^+$-T-lymphocytes (by 12% in the 3rd Group, 25% in the 4th), CD8$^+$-T-lymphocytes (by 23% in the 2nd Group, 24 % – the 3rd, 14%-the 4th) and the number of CD19 increases$^+$- B cells (by 47% in groups 3 and 4).
3. To correct involutional-dystrophic skin changes, the neofibrolifting technique has been improved, including preparatory administration of platelet – rich plasma and after 2 weeks-transplantation of cultured autologous dermal fibroblasts into conditioned skin areas.
4. It was found that as a result of neofibrolifting, acoustic skin density significantly increased relatively the indicators before treatment in patients of Groups 1 and 2 after 12 months by 19% and 33%, respectively; groups 3 and 4 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-20%, dermal fibroblast transplantation-22%, after 6 and 12 months – 22% and 23%, respectively; in people of Group 4 after dermal fibroblast transplantation – 19%, after 6 months – 24 %. Dermal thickness in patients of Group 1 after platelet-rich plasma administration increased by 9 %, dermal fibroblast transplantation-11%, after 6 months – 18%; in women of groups 2 and 3 after 12 months – 28% and 19%, respectively; in people of Group 4 after dermal fibroblast transplantation-16%, after 6 and 12 months-23% and 27%, respectively.
5. Skin hydration in patients of Group 1 after dermal fibroblast transplantation increased by 30%, after 6 months – 26%; in women of Group 2 after platelet-rich plasma administration-17%, dermal fibroblast transplantation – 30%, after 6 and 12 months – 35% and 31%, respectively; in people of Group 3 after platelet-rich plasma administration – 29%, dermal fibroblast transplantation

– 37%, after 6 and 12 months-28% and 29%, respectively; in patients of Group 4 after platelet-rich plasma administration – 13%, dermal fibroblast transplantation – 32%, after 6 and 12 months – 29% and 18%, respectively. Transepidermal moisture loss in Group 1 women after 12 months decreased by 22%; in Group 2 patients after 6 and 12 months-16% and 25%, respectively; in Group 3 patients after dermal fibroblast transplantation-17%, after 6 and 12 months – 28% and 28%, respectively; in Group 4 patients after dermal fibroblast transplantation-13%, after 6 and 12 months – 22% and 19%, respectively.

6. 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 – 50% dermal fibroblast transplantation-79%, after 6 and 12 months-88% and 69%, respectively; in patients of the 4th group after platelet – rich plasma administration – 3.5 times, dermal fibroblast transplantation – 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 the 1st group after dermal fibroblast transplantation increased by 28%, at 6 and 12 months-34% and 40%, respectively; in patients of the 2nd group after dermal fibroblast transplantation – 1.8 times, at 6 and 12 months-2.1 and 2 times, respectively; in persons of the 3rd Group after platelet – rich plasma injection – 1.7 times, dermal fibroblast transplantation-2.7 times, after 6 and 12 months-2.8 and 2.7 times, respectively; in patients of the 4th group after platelet – rich plasma administration – 1.9 times, dermal fibroblast transplantation – 4 times, after 6 and 12 months-4.1 and 3.6 times, respectively.
7. It was found that as a result of neofibrolifting, an increase in the amount of CD4 occurred^{+}-cells, 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 found in younger individuals. CD4 quantity^{+}-cells in patients of Group 1 after platelet-rich plasma administration 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-31%, dermal fibroblast transplantation – 51%, after 6 and 12 months – 76% and 80%, respectively; in people of Group 3 after platelet administration – rich plasma-1.4 times, dermal fibroblast transplantation-1.7 times, after 6 and 12 months – 2.6 and 2.2 times, respectively; in patients of the 4th group after platelet – rich plasma administration – 2 times, dermal fibroblast transplantation-2.5 times, after 6 and 12 months-2.4 and 2.5 times, respectively. CD8 numbers^{+}-cells in women of the 1st group after the administration of platelet-rich plasma decreased by 24%, dermal fibroblast transplantation-47%, after 6 and 12 months – 60% and 43%, respectively; in patients of the 2nd group after the administration of platelet – rich plasma-37%, dermal fibroblast transplantation-44%, after 6 and 12 months – 53% and 49%, respectively; in patients of the 3rd Group after 6 months – 49%; in patients of the 4th group after administration of platelet – rich plasma-39%, dermal fibroblast transplantation – 34%, after 6 and 12 months – 39% and 26%, respectively.
8. Positive clinical, structural, functional and immunological data indicating the anti-aging effect of neofibrolifting are often significant after the introduction of platelet-rich plasma and as a result of subsequent transplantation of dermal fibroblasts with the formation of a stable reliable comprehensive result, which is present 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.

Conflict of Interest: authors do not have any conflict of interest.

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