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Morphological substantiation of application of cellular technologies for correction of striae

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ABSTRACT

The possibility of the application of autologic pluripotent cells from peripheral blood (APCPB) for correction of striae after pregnancy was studied. Visually first signs of improvement of atrophic scars can be noticed in 6-8 weeks after injection of APCPB and gradually progress during 6 months. The atrophy of epithelium, hyperkeratosis, sclerosis of all dermis layers with smoothing of papillae, reduction of number of blood vessels and skin appendages were found before treatment by method of light microscopy. In all observations after injection of APCPB in a connective tissue, which replaces of dermis, an increase of number of vessels at the expense of processes of neoangiogenesis, leukocytic infiltration reduction, thinning and ordering of arrangement of collagen and elastin fibers were detected. These results give a chance for more successful application of cosmetology procedures for correction of these defects of skin.

Keywords: Skin Striae after Pregnancy; Atrophy of Epidermis; Dermal Sclerosis; Pluripotent Cells from Peripheral Blood; Neoangiogenesis

1. INTRODUCTION

Striae distensae or stretch marks—linear atrophic scars, which are localized mainly on skin of mammary glands, stomach, hips and buttocks. At the beginning of the development they are bright pink in color, often with a bluish shade. This color is due to active vascular invasion—a period of fresh stretch marks, which lasts for about 6-12 months. Then there is blanching of tissue, the surface becomes shiny, in texture similar to filigree (cigarette paper). Striae color is lighter than surrounding normal skin because scar tissue contains very few melanocytes and melanin. For the same reason, when the surrounding skin tans, white strips remain [1-3]. From the medical point of view these scars are completely harmless, but they are a widespread cosmetic problem. The treatment of this disease has been a challenge for clinicians and experimenters for a long time [4].

The pathogenesis of striae is still not completely known, and possibly linked to changes in structures that are responsible for elasticity and contractility of the skin. These structures are the components of the extracellular matrix, including fibrillin, elastin and various collagens. In this process the important role plays decrease of expression of genes responsible for formation of collagen, elastin and fibronectin, which, in turn, accompanied by metabolic imbalance of fibroblasts, in particular —metabolism of fibrillin in these cells [5-10]. It should be emphasized that in striae were found signs of chronic autoimmune disorders, in particular the reaction of “graft versus host” [11].

In the time of light microscopy study of striae histopathology were obtained data that well-formed elastin fibers dominate in fresh defects, thickened fibers dominate in the older parts of lesions. Most likely, the elastin fibers in striae were synthesized again, they gradually were thickened and their number increased. According to the data of scanning electron microscopy, unusually dense and well-developed net of elastin with horizontal packing of collagen was discovered. Apparently, the primary target of this pathological process is exactly elastin fibers [12-14].

According to modern representations, physiological regeneration of tissue in adult organism and its reparation in case of damage happens with direct participation of low differentiated cells-precursors or stem cells. The basic source of stem cells is bone marrow, which in addition to the basic function—haemopoetic, is able to generate precursors of cellular elements of a great number of tissues in organism.

Besides bone marrow the pluripotent cells were discovered in other tissues of an adult organism—adiposal, muscular and nerve tissue, and also in peripheral blood

and umbilical cord/placenta blood. Moreover, it is established that depending on a microenvironment the stem cells are able to get through haemopoetic/mesenchymal barrier as possess to high plasticity concerning differentiation and transdifferentiation.

2. AIM

Because of the small efficiency of widely applied techniques of the treatment of striae has been made an attempt to correct this pathology with use of cellular technologies. We demonstrate the morphological description of several cases from practice.

3. MATERIAL AND METHODS

All actions, starting from material-source for receiving of pluripotent cells before the procedure of cellular therapy, were made with observance of existing legal and ethical standards of Russian Federation, and also other standard documents and recommendations concerning this area of medicine. The treatment of striae on stomach skin after pregnancy was performed only after receiving the informed written approval from patients. In our work only autologic cells from peripheral blood were used—as it is more practical and safer as does not demand of selection of matching "donor-recipient".

Isolation of a cellular material from autologic blood received from an elbow vein was performed on the device for cytoplasmapheresis «Haemonetics» (MCS +, the USA). This device uses a make-and-break current principle through centrifuging chamber which was named "bell". There is a division of blood into components in dependence on weight under the influence of centrifugal force. Using this system the device is able to isolate any cellular components from blood at donor or medical apheresis.

Each component leaving a bell passes through an optical linear sensor which allows to define optical density of medium. That makes possible in advance to program separation of specific components of blood, such as plasma, platelets, lymphocytes, granulocytes, stem cells, etc.

The method of flow cytometry was used for the analysis of stem cell population containing in the received material. Samples were analyzed on flow cytofluometer «FACSAria» using FACSDiVa (Becton Dickinson) software.

We used monoclonal antibodies specific to CD34, conjugated with FITC; specific to CD38, conjugated with Cy-Chrome (Becton Dickinson); to antigens to the surface of human cells; isotypical controls to antibodies of classes IgG1, IgG2a mice (Becton Dickinson), conjugated with FITC and PE, accordingly, under the instruction of the manufacturer.

According to the completed analysis on flow cytofluometer using antibodies to superficial human antigens CD34 and CD38, the amount of CD34+ cells was 0.2% and early precursors CD34+/CD38 was 0.1% out of total population of cells. Besides, the quantity of mononuclear cells in 1 ml was counted using trypan blue. We detected that 1 ml of the sample, received after cytoplasmapheresis, has contained 220 × 106 cells.

The received cellular material, containing autologic pluripotent cells from peripheral blood (APCPB), was entered directly after separation procedure into zones of skin atrophy using following method:

1) Suspension of APCPB was collected into a sterile syringe in capacity of 5.0 ml with the rubber pump. The stroke of the syringe pump should be tight but smooth.

2) Before injection the skin was processed by a napkin made of notwoven material wetted with 0.5% water solution of chlorhexidine.

3) A needle on a syringe replaced with a needle 30G. Injection was performed in zones of striae linearly retrogradually. A needle was entered on all length strictly by cut upwards or cut downwards A needle was entered on all length strictly by cut upwards or cut downwards. The needle cut should not be placed sideways, that not to injure the skin. During removal of the needle out of the tissue the pressure upon the pump was made, for filling skin atrophy by plasma containing autologic AHPCPB. During procedure the needles were replaced several times when they became blunt. Anesthesia is not necessary, as the procedure is a little painful (diameter of the needle is only 0.3 mm).

The samples of skin (epidermis and dermis) size 2 × 2 mm from the front surface of the stomach on border of normal skin and striae after pregnancy were preserved in a 4% paraformaldehyde on biphosphate buffer (pH 7.4) for at least 24 hour, dehydrated in a gradien of ethanol, lightened in xylene and embedded in histoplast. Microscopic sections of 5-7 microns thick were stained with hematoxylin and eosin, and studied under a light microscope Axioimager M1 (Carl Zeiss, Germany) with a magnification of up to 1200 times.

4. RESULTS

Patient O., 28 years old, (pregnancy was 4.5 years ago), visually the first signs of improvement of atrophic cicatrices appeared in 6-8 weeks after the injection of AHPCPB. In 6 months filling striae from inside was well visible (**Figures 1-4**). In the first place we noticed that the color of striae approached the color of normal skin. Thus "old" atrophic striae which did not acquire sun tan, approached the color of normal skin and started to acquire sun tan (**Figure 2**). The depth of tissue retraction decreases. At this stage the changes of skin were visible.

If initially skin was atrophic, thinned, at compression easily gathers in folds and looks like wrinkled cigarette paper (**Figure 3**), after 6 months it became more dense,

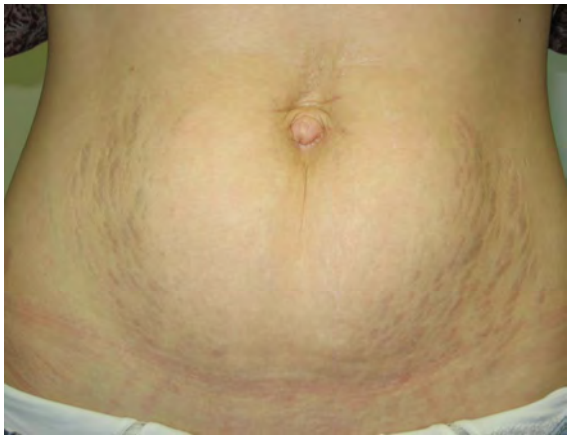

Figure 1. Abdominal skin of the patient O. with numerous linear atrophic scars before treatment.

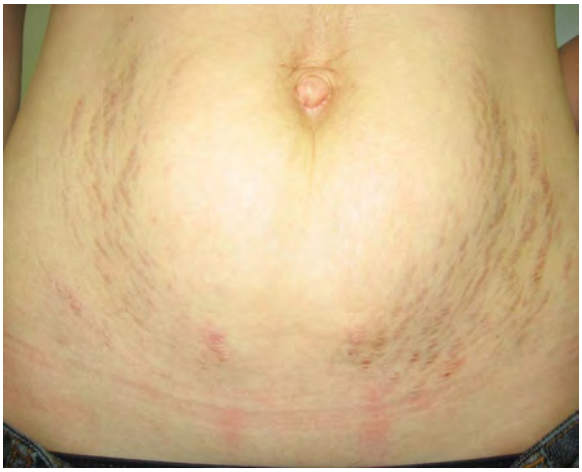

Figure 2. Status of stretch marks in 6 months after the introduction of APCPB. The color of the stretch mark is much closer to the color of the surrounding skin, in some areas there is pigmentation as a result of sun tan.

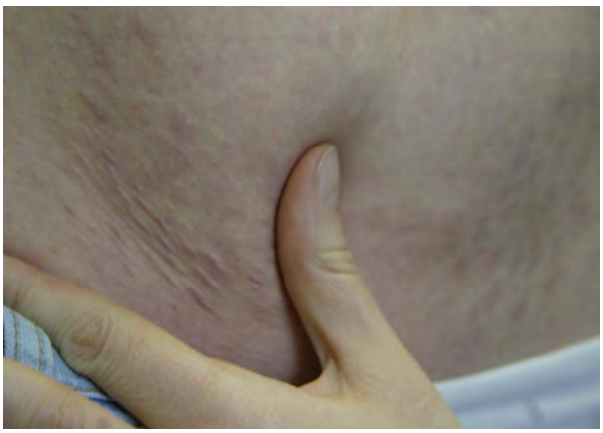

Figure 3. Abdominal skin with stretch marks after pregnancy before using APCPB. Skin is atrophic, thinned, at compression easily gathers in folds and looks like wrinkled cigarette paper.

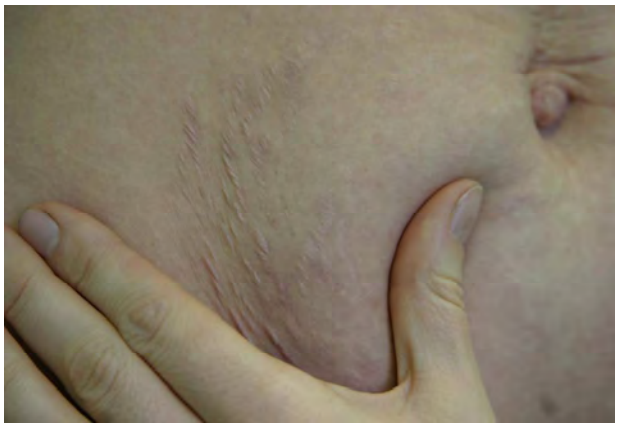

Figure 4. 6 months after using AHPCPB the skin becomes more dense, looks thicker, the striae become narrower.

looked thicker. Perhaps, because of the improvement of surrounding skin the striae looked narrower (**Figure 4**).

In the time of histological study of striae of this patient, which was taken before the procedure of treatment by APCPB, were noted an atrophy and thinning of epithelium, hyperkeratosis, flattening of papillae and smoothing of papillary layer in dermis, and almost total absence of reticular layer which was replaced with connective tissue with a chaotic arrangement of fibers and a small amount of blood vessels with perivascular leukocytic infiltration with prevalence of lymphocytes and macrophages. The type of vessels cannot always be identified due to expressed sclerotic and inflammatory changes (**Figures 5, 6**).

In the study of biopsy from the edge of the defect within 1 month after the injection of APCPB were also found the above mentioned changes (atrophy of the epithelium and hyperkeratosis, flattening and smoothing of the dermal papillae, sclerosis of the reticular layer). However, the severity of sclerotic changes in the dermis was somewhat less, there was formation and order of collagen fibers in the reticular layer, where was significantly more blood vessels and drastically was reduced degree of perivascular leukocyte infiltration. Also were found young blood vessels with very thin walls, it was not always possible to trace the endothelial line. Red blood cells were present not only in these vessels, but were also located around vessels (**Figures 7, 8**). These newly formed blood vessels were not even remotely similar to the granulations, and therefore the assumption of the induction of inflammation in tissues after APCPB injection should be rejected.

4 months after the injection of APCPB the atrophic changes in the epidermis became more pronounced, completely vanished skin appendages and smoothed papillae, but one can note the appearance of melanocytes. Signs of edema were more significant, there were cavi

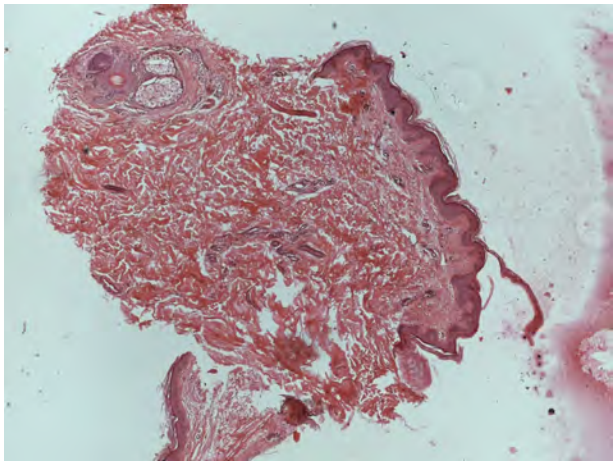

Figure 5. The results of morphological study of stretch marks of the patient O. before treatment. Atrophy and thinning of the epithelium, hyperkeratosis, flattening of the papillary layer in dermis. Reticular layer is replaced by connective tissue with randomly distributed fibers and a small amount of blood vessels with perivascular leukocytic infiltration. Hematoxylin and eosin. Magnification × 40.

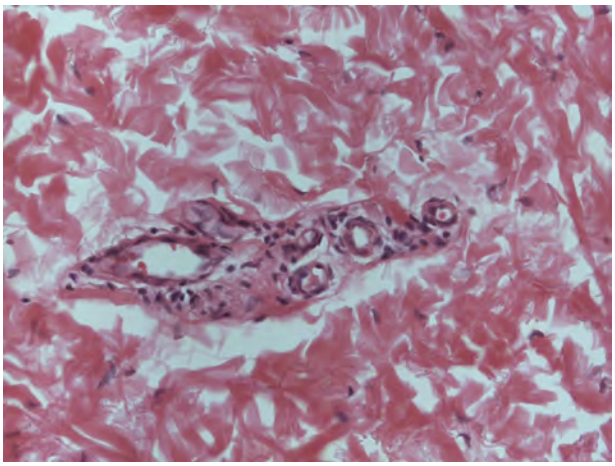

Figure 6. The fragment of **Figure 5**. Chaotic arrangement of fibers in the intercellular matrix of connective tissue in dermis. In cytogram of perivascular leukocytic infiltrates the lymphocytes and macrophages are dominate. Magnification × 400.

ties with fluid, an expansion of the components of the lymphatic vessels was found. Scar connective tissue in the reticular layer of the dermis looked friable. The number of vessels decreased slightly, but still remained more than in initial conditions. The vessels became larger and sclerosis of their walls can be noted. By that time there were again appeared perivascular leukocytic infiltration with a predominance of lymphocytes. Young vessels with thin walls were still present in the dermis (**Figures 9, 10**).

In skin biopsy of patient M., 22 years old (pregnancy 2.5 years ago), before treatment were found atrophy of

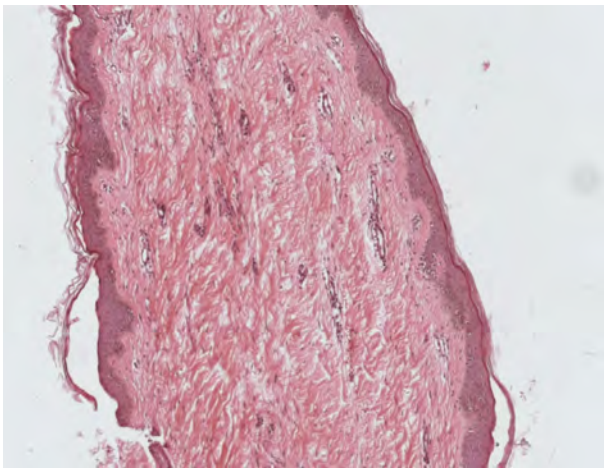

Figure 7. Biopsy from the edge of the defect within 1 month after the injection of APCPB: atrophy of the epithelium, hyperkeratosis, flattening of the dermal papillae, appendages of the skin are absent. The severity of sclerotic transformation of the reticular layer of the dermis is reduced, the fibers of intercellular substance became thinner and arranged in parallel. The number of vessels increased. Hematoxylin and eosin. Magnification × 40.

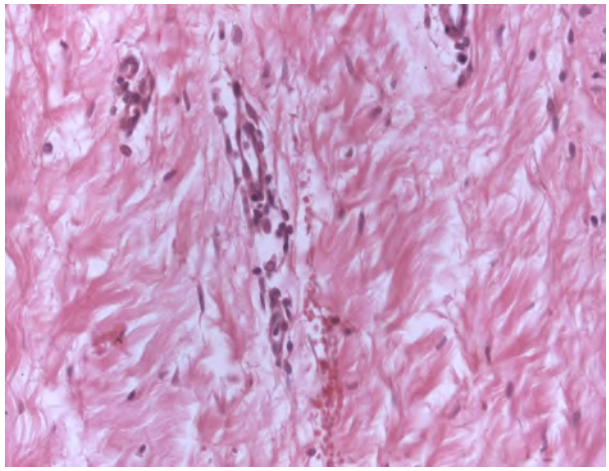

Figure 8. The fragment of **Figure 7**. Drastic reduction of perivascular leukocytic infiltration. In the connective tissue the newly-formed blood vessels with thin walls are present , where nuclei of endothelial cells are very rare. Red blood cells are located not only in vessels but also perivascular. Magnification × 400.

the epithelium, hyperkeratosis, and almost complete absence of all layers of the dermis—papillary and reticular, and complete absence of skin appendages: hair follicles, sudoriferous and sebaceous glands. The dermis was replaced with scar connective tissue with edema and a small amount of blood vessels, around which the perivascular leukocytic infiltration with a predominance of lymphocytes and a large number of neutrophils was present.

In the study of biopsy specimen from the edge of the

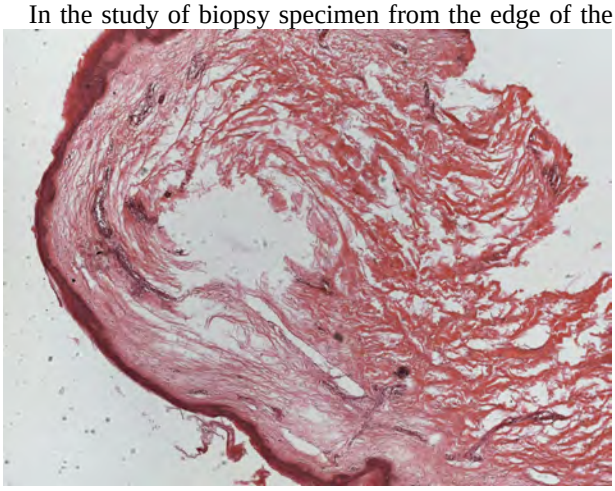

Figure 9. Striae area in 4 months after injection APCPB. Atrophic changes and hyperkeratosis of the epidermis are more pronounced. Skin appendages vanished completely and papillae are smoothed. Instead of scar connective tissue in the reticular layer of the dermis there is friable connective tissue where significant signs of edema and expansion of the components of the lymphatic vessels are noted. The number of vessels is decreased, but the vessels became larger and have sclerosed walls with perivascular leukocytic infiltration. Hematoxylin and eosin. Magnification × 40.

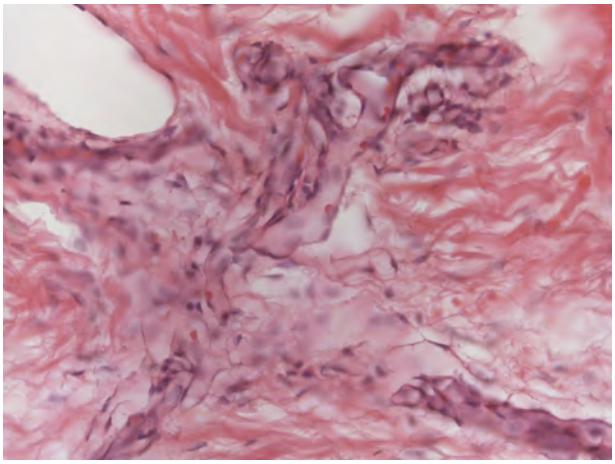

Figure 10. The fragment of **Figure 9**. The newly-formed blood vessels with thin walls and red cells located in the connective tissue on the place of reticular layer of dermis. In these vessels the endothelial lining can be seen along the full length, the remaining layers of the wall (muscle, adventitia) are absent. Magnification × 400.

defect 6 weeks after the introduction of APCPB were also found atrophy of the epidermis and hyperkeratosis, but the severity of atrophy was significantly less. Also skin appendages and all layers of the dermis were absent. However, there was a formatting and some ordering of collagen fibers in the connective tissue on the place of dermis, there was significantly more vessels, reduced the severity of edema and the degree of perivascular leukocyte infiltration. In cytogram of such small infiltrates the lymphocytes and macrophages dominated.

In 5 months after treatment, sclerosis of the dermis was still decreased. The number of vessels decreased, but the remaining vessels were large. Leukocytic infiltrates around the vessels were present again and appeared signs of edema.

Patient S., 30 years old (pregnancy 9 years ago): in skin before the injection of APCPB were found hyperkeratosis, atrophy of the papillary and reticular layers of the dermis and smoothing, to almost complete disappearance, of dermal papillae. Also hair follicles, sudoriferous and sebaceous glands were absent completely. The dermis was replaced by scar connective tissue with a chaotic arrangement of thick fibers and large cavities with a transparent content and a small amount of blood vessels, around which were noted perivascular edema and leukocytic infiltration with a predominance of macrophages.

In the study of biopsy specimen from the edge of the defect in 6 weeks after the injections of APCPB there were found almost normal skin structure—epidermis, papillary and reticular layers of the dermis. In this case, only hyperkeratosis, small lymphocytic-macrophage infiltration of the vascular walls, a moderate perivascular and tissue edema attracted attention. Also the number of small vessels increased.

5. DISCUSSIONS

Thus, all patients had similar initial state of skin striae after pregnancy, (of course, the severity of the skin changes was different) and was consistent with the literature data devoted to the description of “old” striae, which are characterized by the thinning of the connective tissue stroma, which leads to the formation of lines of atrophied skin, and the presence of a large number of thickened elastin fibers [5,6,8,13].

Due to thinning and sclerosis of the dermis, which remains over a long period of time, its blood supply is disturbed. As a result of vascular disturbance there is no proper nutrition and oxygenation of cells. There are formed leukocytic infiltrates for lysis of nonviable tissue. Scar changes gradually progress, and all structures of the dermis, as the result of fibroblasts functional disturbance [5-10,13], with time are replaced by randomly arranged thick fibers of collagen and elastin.

After the introduction of APCPB the edema of the dermis and atrophy of the epithelium may become stronger or weaker. However, after the procedure, in all observations in the connective tissue on the place of dermis there is an increase of number of vessels, thinning and ordering of distribution of collagen and elastin fibers.

Apparently, injected cells somehow stimulate the growth of blood vessels, perhaps participate in this process because of the presence of pluripotent cells which were already stimulated to differentiate into endothelial or pericytal directions. After increasing the number of vessels, especially the "young" with thin walls, metabolic processes in the diseased tissues are improve. As a result of optimizing conditions of life and functioning of the fibroblasts and tissue leukocytes the metabolism of extracellular matrix components of the connective tissue of the dermis intensifies, there is a rejuvenation of collagen and elastin fibers, which is manifested in the appearance of finer structures and ordering their location [13]. Also, when metabolism improves and therefore the volume of nonviable tissue reduces, perivascular infiltration and its activity are decreased.

It should be noted that in the remote period of up to 5 months after the introduction of APCPB the number of vessels somewhat is decreased, but their size still remains bigger than original. The processes of neoangiogenesis at this time does not stop and is quite active, evidence of that can be the presence of wide blood vessels with thin walls even at this period.

As a result of these therapeutic procedures some positive aesthetic results were obtained due to the fact that stretch marks appear not as "sunken, hollow", become narrower. In addition, the state of the surrounding skin improves, it becomes denser. Making this procedure, we did not attempt to achieve a significant therapeutic effect, which manifests itself in full or partial disappearance of skin defects. Use of cellular technology improved the microcirculation in "old" stretch marks. The scars, existing for many years, according to the morphological pattern following the introduction of APCPB, in particular, in number and size of vessels, in appearance fibers of extracellular matrix of the dermis, are similar to "young" scar.

After rejuvenation of the stretch marks it is possible to perform some cosmetic procedures (mechanical, chemical or laser microdermabrasion (grinding) of the skin), aimed at making these scars to less visible or even completely to eliminate them, as only fresh stretch marks, which appeared less than a year ago, can be corrected effectively [15,16].

Despite the fact that there are possibilities to struggle against stretch marks such as laser grinding, and peeling with the use of corrosive acids and microdermabrasion, as well as their combinations, the prevention of stretch marks is difficult process, but this is more effective than treatment of already existing stretch marks [17-19].

6. CONCLUSIONS

Thereby, in abdominal skin striae after pregnancy, completed several years ago, were found atrophy of the epithelium, hyperkeratosis and sclerosis of all layers of the dermis with smoothing of papillae, the decrease of number of blood vessels and skin appendages. After the introduction of APCPB the first visual signs of improvement of appearance of atrophic scars were detected in 6-8 weeks. In 6 months there is well visible the filling of scars from the inside, the color of stretch marks become closer to the color of normal skin, the "old" atrophic scars begin to get sun tan. The depth of the sunken tissue is reduced, scar skin becomes more dense, less thinned on appearance. According to morphological data after the application of APCPB the edema of the dermis and atrophy of the epithelium may become stronger or weaker. But in all cases in the connective tissue on the place of dermis there is increasing of number of blood vessels due to the process of neoangiogenesis, reducing the degree of leukocytic infiltration, thinning and ordering distribution of collagen and elastin fibers, which enables more successful application of cosmetic procedures for the correction of skin defects.

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