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([*] - in our country a two-stage system for obtaining academic degree has been adopted, i.e. Doctor of Science is the highest research degree, many scientists having D.Sc. are Professors; Ph.D degree is the equivalent to doctoral candidate)

ABSTRACT

Background. Progressive development and significant advances in the field of cellular biology and technologies, developmental physiology and gene engineering resulted in the formation of a new era and branch of medicine — regenerative medicine. This new branch includes science-based and safe approaches for creating new cells and tissues and restoring the structure and functions of damaged ones. The objective of the regenerative medicine is to replace or regenerate human cells, tissues, or organs. Such a replacement helps to recover the normal function that has been lost. An important change in the paradigm of regenerative medicine is the switching to a cellular therapy. In this investigation, the authors studied two surgical methods for the treatment of vitiligo and conducted a comparative analysis of post-treatment repigmentation.

Research objective: to conduct a comparative analysis of cell transplantation methods in the treatment of vitiligo.

Materials and methods. In this research patients with vitiligo underwent a surgical treatment. Depigmented patches were studied according to the localization of the pathological focus. The transplantation of non-cultured epidermal cells was performed in 63 patients. The transplantation of a mixture of hair follicle cell suspension (NCECS+NCORSHFS) obtained from the scalp of a patient with vitiligo in combination with the mixture of non-cultured epidermal cell suspension (NCECS) was performed in 50 patients. Before transplantation, the prepared suspension was injected into the depigmented patch by mesotherapy. Besides, the authors conducted a comparative evaluation of repigmentation indicators and treatment efficacy.

Findings. The performed method of surgical treatment for vitiligo is highly effective, confirmed by the absence of depigmentation signs immediately on the treatment completion. The recommended treatment course is 1-3 procedures, depending on the location and scope of the pathological process. The use of this method will contribute to efficacy improvement of vitiligo treatment.

Key-words: transplantation, hair follicle cell suspension, automelanocytes, repigmentation.

INTRODUCTION

Over the last few years, due to the development of regenerative medicine much attention is paid to the methods of epidermal-dermal autotransplantation, based on the transplantation of cell grafts containing functional melanocytes and melanocytes-precursors in the depigmented patches. In modern practice, different methods of transplantation of both cultured and non-cultured cell and tissue grafts are being applied [1]. When performing these methods, separation of keratinocytes and melanocytes is performed (by trypsinization and centrifugation) on skin flaps taken from the donor areas containing healthy functioning cells. The resulting cells are transplanted in the form of a suspension into recipient ones, as the depigmented area are cultivated in order to create a culture of epidermal cells or a pure culture of melanocytes. In the process of cell culture various media containing antibiotics, growth factors, bovine serum with the addition of patient serum are used in a special high-tech sterile apparatus [2; 3].

The main advantage of the transplantation methods is the possibility of treating large-area vitiligo lesion using small fragments of donor skin. The disadvantages include the complexity of the procedures and possible failures during cell culture [4]. There are several methods of cell transplantation:

1. Transplantation of non-cultured epidermal cell suspension. The method consists in obtaining a suspension of epidermal cells (keratinocytes and melanocytes) from small dermo-epidermal layers of the skin, which is transplanted into the depigmented patch [5].
2. Transplantation of cultured epidermal grafts. Application of this method includes using of a dermatome, when a small skin flap is taken from the donor area, which is subjected to trypsinization. After separation of the epidermis from the dermis, the cell suspension is obtained. Subsequent manipulations are possible with either unchanged suspension or a culture of melanocytic cells isolated from it. The suspension is cultivated for several weeks, then transferred to a special support in the form of the epidermal membrane. The use of a special base should provide the most favorable conditions for the

vital activity of cells, which reduces the time of repigmentation and makes it the most complete [6; 7; 8].

3. Transplantation of cultured autologous melanocytes. The method involves isolation of functionally active melanocytes from the graft taken from normally pigmented skin, which are cultured within 15–30 days in order to obtain a pure culture. The grown culture of melanocytes is distributed in the recipient zones over the surface of the depigmented skin (the density of distribution can reach 1000–2000 melanocytes/mm^2). However, this method is considered to be much expensive, as requires a specially equipped high-tech laboratory and qualified personnel [9; 10; 11].
4. Transplantation of non-cultured extracted hair follicle outer root sheath cell suspension. This method began to be applied in the clinical practice relatively recently. It is based on the use of cells of the outer root sheath of hair follicles as a source of repopulation of active melanocytes and their precursors. Hair is taken from the skin of the occipital region of the scalp and only in the anagen phase. The use of such technology allows avoiding the growth of unwanted hair in the areas of transplantation and long-term manipulations with cells in vitro, as well as obtaining a significantly larger number of active melanocytes in comparison with other methods of transplantation. According to published data, the transplantation of 20 follicular units affords to recover pigmentation in the depigmented area of 25 cm^2 [12].

MATERIAL AND METHODS

All patients with vitiligo involved in the research underwent a surgical treatment. Depigmented patches were studied according to the localization of the pathological focus. The transplantation of non-cultured epidermal cells was performed in 63 patients. The transplantation of a mixture of hair follicle cell suspension (NCECS+NCORSHFS) obtained from the scalp of a patient with vitiligo in combination with the mixture of non-cultured epidermal cell suspension (NCECS) was performed in 50 patients. Before transplantation, the prepared suspension was injected into the depigmented patch by mesotherapy

In our research we propose to apply the method of transplantation of a suspension of non-cultivated cells of the outer root sheath of hair follicles in combination with non-cultured automelanocytes. The working principle of Regenera Activa apparatus is based on clinical trials determining the existence of a high concentration of the obtained cells in solid tissues. Cells and other precursor elements are concentrated using a calibrated mechanical process and filtrate. The device allows to obtain a cell suspension, which can be used for tissue regeneration; in our case, a suspension from hair follicle grafts containing

mesenchymal cells of melanocyte precursors. The technique is following: one or two tissue samples are placed in the apparatus (Kit Regeneracons) together with sterile physiological serum (1.5 ml). Treatment of a hair follicle sample can take 2 to 4 minutes depending on the number of holes made. During the degradation process, the cells pass through the filter and liquid suspension and work to the bottom of kit Regeneracons.

Small skin flaps, with reference to the size of the depigmented (1 cm^2 of donor epidermis is planned for 10 cm^2 of the depigmented patch) area, taken from the donor zone, undergo the process of trypsinization (kept for 20 minutes in trypsin solution). After enzymatic cleavage, the epidermis is mechanically separated from the dermis, then centrifuged and, as a result of the step-by-step procedures, a suspension of keratinocytes and melanocytes is obtained. Depigmented patches are dermabrased by a dermabraser, and the mixed ready-made suspension in the form of application is fixed for 7 days.

A method for preparing a cell suspension using Regenera. Using the high-technology apparatus Regenera (Italy), the extraction of mesenchymal cells of the hair follicle is carried out and transplanted in combination with functionally active melanocytes in the depigmented skin areas of vitiligo patients. This technology has a highly specific effect on damaged tissues and a targeted complex regenerative stimulation on a degrading complex of cellular and protein structures of the restored tissues. The technology of highly specific stimulation of the degrading tissues is absolutely safe, since the patient's own tissues are used. It is easy to perform in the procedure room (class B), does not require a long-term physician's training, has a minimum of contraindications and a long lasting effect. This technology can be applied not only in the treatment of vitiligo, but also in a wide range of other diseases of great social importance, such as diabetic foot, trophic ulcerative skin lesions, degenerative changes of soft tissues, dermal cicatricial changes, various types of alopecia and other diseases associated with degenerative and trophic tissue disorders. The technology is based on the isolation of the autologous vasostromal cell fraction obtained from micrografts (bioptates) of healthy areas (hair follicles) of the patient, prepared directly during the procedure by disintegration, separation and isolation of mesenchymal autologous cells and introducing into the area requiring recovery, in the form of the suspension. Mesenchymal cells act as a stimulant for the recovery of degraded tissue. The resulting suspension consists of cells of the regenerated tissue and saline solution. The proposed technique is more modernized in comparison with the currently existing one. The innovative technology is based on a fundamentally new method of isolating the cell suspension, obtained transplant from the autologous

vasostromal micrograft, as well as on specialization and stimulation of the degrading tissue that requires recovery. The method consists in administration of the suspension of a disintegrated and separated pool of mesenchymal cells enriched with growth factors specific for the tissue into the depigmented patch, as well as specialization and stimulation of regeneration, which allows to recover not only lost cells, but also the entire tissue association of the affected tissue.

The technique of carrying out. Using a medical punch 2-3 holes (3.0 mm) are made to take a small tissue sample from the donor intact scalp, preferably from the occipital region (Fig. 1). One or two tissue samples are placed together with sterile physiological serum (1.5 ml) into the kit Regeneracons (Fig. 2), which is installed in the Regenera apparatus. So the process begins, which can last 2 to 4 minutes depending on the type of tissue and the result required to obtain the suspension of cells and stromal tissue. The degradation time can be standardized for each type of tissue. During the degradation process, the cells pass through the filter and liquid suspension and work to the bottom of the kit Regeneracons. When the cell degradation process is complete, the kit Regeneracons can be removed from the Regenera apparatus. The resulting tissue under maximum sterile conditions is processed by the Regenera apparatus, which allows to dissect the tissue and filter mesenchymal cells, which are immediately introduced into the pathological tissue of vitiligo patients (Fig. 3).

Surgical treatment method of vitiligo with non-cultivated keratinocyto-melanocytic suspension with hair follicles is carried out by transplantation of autologous non-cultivated epidermal cell suspension in combination with dermal cell suspension with active factors of hair follicles using Regenera-Active. Cells and other precursor elements are concentrated during mechanical process. This process allows to obtain the cell suspension which can be used for neogenesis; in this case the suspension made of hair follicle grafts containing mesenchymal cells of the melanocytes precursors is used. The advantage of the method consists in isolation of non-cultivated melanocytes from the epidermis cells (Fig. 4) and their administration into the depigmented patch (Fig. 3).

Non-cultivated epidermal cells of keratinocytes-melanocytes are taken by small flaps from the skin surface, with reference to the size of the depigmented (1 cm^2 of donor epidermis is planned for 10 cm^2 of depigmented patch) focus taken from the donor area; underwent the process of trypsinization (kept in trypsin solution for 20 minutes) (Fig. 5). After enzymatic cleavage, the epidermis is mechanically separated from the dermis, then centrifuged and, as a result of the step-by-step procedures, the suspension of keratinocytes and melanocytes is

obtained. Depigmented patches are dermabrazed by the dermabraser, and the mixed suspension in the form of the application is fixed for 7 days.

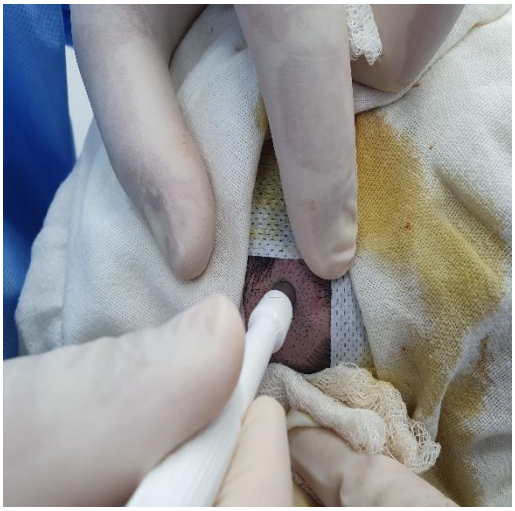

Fig. 1. Taking of grafts from the occipital region of the scalp

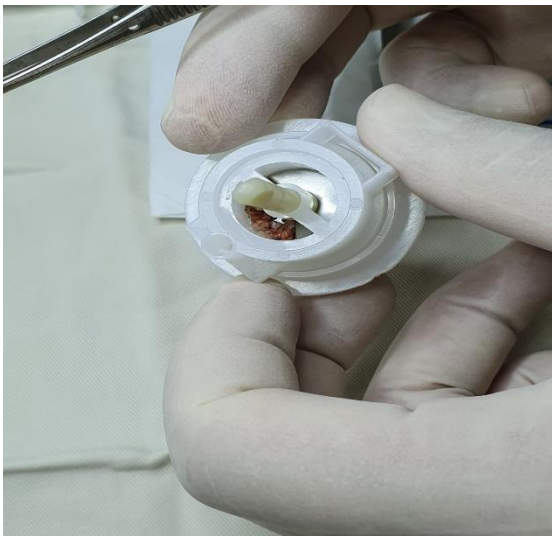

Fig. 2. Bioptate-grafts of hair follicles in the kit Regeneracons

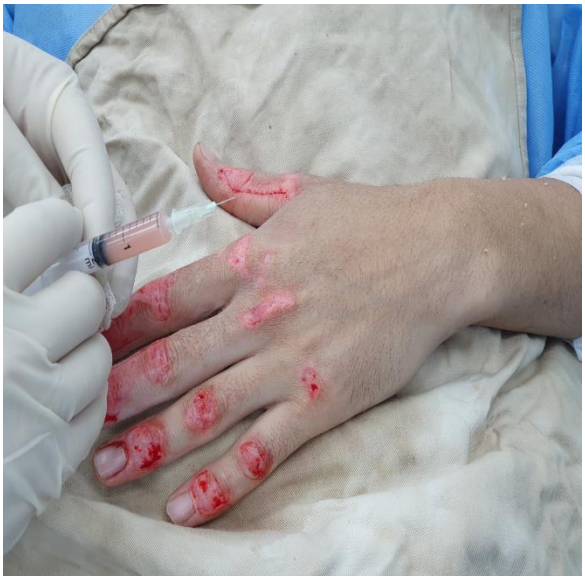

Fig. 3. Mesotherapeutic administration of the hair follicles suspension into the depigmented patch after dermabrasion

Fig. 4. Non-cultured epidermal cell suspension (NCECS)

Fig. 5. Epidermis from donor skin in trypsin solution

The patients underwent the combination of NCECS + NCORSHFS treatment method, using Regenera-Active. Repigmentation process began 3 weeks after the treatment in 10% of cases, after 4 weeks it was 25%, and 8 weeks - over 80%. The clinical pattern after the NCECS method after 6 months showed an increase in the repigmentation level by 59.27 ± 3.28% in case of non-segmental vitiligo, whereas in case of segmental vitiligo improvement in pigmentation was noted by 66.82 ± 3.52%. The combined method NCECS+NCORSHFS resulted in the increase in repigmentation indicator by 84.60 ± 2.21% in non-segmental vitiligo and by 91.00 ± 1.45% in segmental form of the disease (P <0.001) (Table 1).

Table 1

Segmental		**Non-segmental**	
After NCECS	After NCECS+NCORSHFS	After NCECS	after NCECS+NCORSHFS
66,82±3,52%	91,00±1,45%	59,27±3,28%	84,60±2,21%
P<0,001		P<0,001	

Evaluation of efficacy of surgical treatment using the method of non-cultivated keratinocyto-melanocytic suspension in vitiligo patients according to the localization of the disease process (Table 2).

Table 2

Repigmentation level when using the NCECS method, n=63			
Face, n=24	Trunk, n=13	Hands, n=17	Legs, n=9
75,2±3,38	58,85±4,43	51,47±5,39	50,55±5,29

Note: p - reliability of the data compared with the indicators before treatment * - p <0.01

Evaluation of efficacy of surgical treatment using the method of non-cultivated keratinocyto-melanocytic suspension in combination with hair follicles in vitiligo patients by localization of the disease process (Table 3).

Table 3

Repigmentation level when using the NCECS + NCORSHFS method, n=50			
Face, n=24	Trunk, n=13	Hands, n=17	Legs, n=9
86,84±4,61	85,0±5,68	85,6±5,29	85±9,79

Note: p - reliability of the data compared with the indicators before treatment * - p <0.01

Table 4

Method	**Indicator of repigmentation (%)**
After NCECS, n=63	63,17 ± 2,47%*
After NCECS + NCORSHFS, n=50	85,88 ± 1,82%*
*P< 0,001	

When evaluating the overall treatment efficacy, the repigmentation indicator averaged 63.17±2.47% in 63 patients who underwent NCECS, and 85.88±1.82% in 50 patients treated with the NCECS + NCORSHFS method (P <0.001) (Table 4).

The comparative evaluation of the surgical transplantation methods for vitiligo showed a high treatment efficacy of the method of non-cultivated keratinocyto-melanocytic suspension in combination with hair follicles, which was characterized by a significant increase of repigmentation level by 1.36 times compared with the method of non-cultivated keratinocyto-melanocytic suspension in vitiligo patients (P <0.001).

The possibility to eliminate the cosmetic defect in a short time significantly improves the quality of life of patients, contributing to the improvement of their psychological state and social adaptation.

CONCLUSION

Thus, we may conclude that the performed method of surgical treatment of vitiligo is highly effective, which is confirmed by the absence of signs of depigmentation immediately on the treatment course completion. The recommended treatment course for this surgical method is 1-3 procedures, depending on the location and scope of the pathological process. Using of this treatment method contributes to achieve favorable treatment outcome for vitiligo as well as to increase the duration of the long-lasting effect.

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