Application and evaluation of the effectiveness of control signals of connective tissue proliferation in models of therapy for acne and age-related skin changes

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Introduction

The connective tissue of the human body plays an important role in its development, and the role of a key indicator in its aging. From the point of view of cosmetology, we can say that a person's age is determined by the age of his connective tissue. Slowing down the aging of connective tissue is one of the most important tasks of rejuvenating not only the skin, but the whole organism as a whole, and solving aesthetic problems.

The main cellular elements of connective tissue are fibroblasts. These cells of mesenchymal origin are the main cells of the middle connective tissue layer of the skin called the dermis. The main function of dermal fibroblasts is to participate in the metabolism of the intercellular substance. Condition and function of major extracellular componentsconnective tissue, collagen, elastic and reticular fibers and extracellular matrix depend on the activity of fibroblasts. Skin fibroblasts synthesize and secrete a large number of biologically active substances: various growth factors (KGF, EGF, FGF, TGF), components of the extracellular matrix (glycosaminoglycans, hyaluronic acid, chondroitin sulfate) and enzymes. With age, the number of fibroblasts in the skin and in the connective tissue of various organs and systems of the body decreases, they become less active, the thickness of the dermis decreases, the moisture content decreases, as a result elasticity and firmness are lost, as a result of which it developsvisible aging of the skin and at the same time, a certain class of age-related damage to internal organs. Visible aging of the skin in different parts of the body is uneven, open areas and skin in places of natural folds age faster. One of the main causes of aging is considered

decline ability of skin fibroblasts to division and a decrease in the level of their synthetic activity. By stimulating these processes, it is possible to achieve a significant slowdown, and sometimes reversal of the visible aging process.

Another problem in cosmetology is diseases, as a result of which pathological processes in the connective tissue develop in a patient at a young age (often in the pubertal period), but are difficult to reverse development. Such diseases include acne ("acne") - a polymorphic, multifactorial disease of the sebaceous glands and sebaceous hair follicles of an inflammatory and obstructive nature.

Acne treatment with orthodox methods:

- as a rule, long-term and complex, even with very mild forms of the disease, and requires a lot of patience on the part of the patient and the attending physician,
- leave a sufficiently large surface of damage to the skin of the face, and therefore should supplemented by long-term, harmful health patient and, generally, ineffective cosmetic procedures aimed at the condition of the skin. on the improvement

Many of the modern methods used in cosmetology, stimulate own skin cells, in particular fibroblasts. An increase in the activity of fibroblasts and an increase in their number can be achieved with the help of transplantation of autogenous fibroblasts to the areas of the skin where it is necessary. Before the real possible transplantation of autogenous fibroblasts, many methodological issues and safety issues of such procedures were solved [1]. Progress in this direction was achieved when they learned to cultivate (maintain viability) fibroblasts outside the body (in vitro). But even under optimal conditions in vitro human embryonic fibroblasts are able to divide a limited number of times (50 ± 10). The last phase of cell life in culture was defined ascellular aging, and the phenomenon is called the "Hayflick limit" [2]. Normal fibroblasts in culture retain the diploid karyotype, are able to grow only in a state attached to the surface of the culture flask, exhibit the phenomenon of contact inhibition and have a limited lifespan, they are not oncogenic and have low expression of histocompatibility antigens [3]. Scientific research and clinical development in this direction is very intensely, which is associated with the general development in the world of cellular technologies. Now you can use

fibroblasts cultured outside the body for the production of immunobiological medicines and therapeutic purposes. The use of cell cultures in the world of cosmetology began relatively recently, with the use of both allogeneic (foreign to the body) and autogenous fibroblasts [4]. The results of world experience indicate the effectiveness of using one's own cells both for slowing down the process of visible aging of the skin and improving its general condition, and for restoring the functions of organs and body systems damaged as a result of aging. The duration of the preservation of the effect on the condition of the skin according to the results of world practice is up to 5-7 years after transplantation of autogenous cells ("Isolagen" USA). The explanation for this phenomenon is, possibly, in the fact that during the cultivation of autogenous fibroblastsin vitro their reactivation ("rejuvenation") occurs, that is, a return to a state close to the nature of mesenchymal stem cells. The cell culture obtained after trypsinization of the biopsy material contains both "young" and "old" cells. All these cells are placed in a medium containing embryonic serum, that is, in conditions somewhat similar to those existing in the embryonic state. At the same time, there is an active stimulation of the proliferation of "young" cells that have retained a high capacity for growth and division, and dilution and washing out of the culture of "old" cells that have lost the ability to proliferate. When transplanting back to a patient, such a reactivated cell culture: actively populates the dermis, intensively synthesizes the entire complex of components of the extracellular matrix and growth factors [4]. But not always the process of transplantation of autologous fibroblasts,

purpose of work

1. Apply the control signal of cell culture (fibroblast) death for therapy acne and age-related skin changes.

2. To assess the degree of efficiency of control signals of tissue proliferation fibroblasts as a factor of repair of damaged connective tissue of a patient in models of therapy for acne and age-related changes.

Hypothetical model of the control signal

Presumably, there are two classes of control signals (CS) that regulate the proliferative processes of the connective tissue of the body:

1. The first class of RS represents, from a systemic point of view, information that in an active process of proliferation of connective tissue occurs in the body, accompanied by the formation of fibroblasts and stem cells, their mitosis and their replacement of damaged cells (or parts) of the connective tissue. In this case, the natural response of the organism will be a comprehensive adaptation of the organism as a whole to the course of integral adaptation and its subsequent stabilization. It is natural to assume that the basic signal of this type is the issued CSbreeding (living) fibroblasts.

2. The second class of CS is a signal that informs the body about cell death connective tissue and fibroblasts, in this case it can be assumed that the body's natural response to the signal will be an increase in the reproduction of its own fibroblasts. Note that this process, by its definition, is not equilibrium, it does not provide for a state of stabilization of the connective tissue, its basic signal isdeath signal fibroblasts.

Due to the difference in the hormonal background of the male and female body, it can also be assumed that the CVs of fibroblast proliferation for them may be different in their structure.

Materials and methods

1. Obtaining control signals for connective tissue repair

To record the CA was used computerized apparatus for electropuncture diagnostics, drug testing, adaptive bioresonance therapy and electro-, magnetic and light therapy for BAP and BAZ "IMEDIS-EXPERT" (registration certificate No. FS 022a2005 / 2263-05 of September 16, 2005) [5]. The recording was made using a light probe and an inductor connected to the input of the "IMEDIS-EXPERT" apparatus operating in the "Transfer" and "Medication testing" modes. The knob of the potency (amplification) regulator of the apparatus signal was at the same time in position "7" (entry "one in

one").

Human fibroblast cell cultures were used to generate a signal for fibroblast repair. Obtaining and cultivation of fibroblasts from a biopsy specimen was carried out according to the standard method [6]. During cultivation, a medium containing 90% of the DMEM nutrient medium was used.4 and 10% fetal calf serum, cloning of fibroblasts, trypsinization with Versene solution and trypsin at 4 C. Then the suspension of cells was plated on plastic Petri dishes and

incubated under saturating humidity at 37 C, with 3-5% CO_2.

As control signal of fibroblast repair electronic recording the sum of Korsakov's potencies (3, 6, 8, 10, 12, 15, 30) of the preparations for the death of fibroblasts, made on the 7th day after opening the Petri dishes with culture.

2.Evaluation of the degree of efficiency of control signals of tissue proliferation of fibroblasts

The following models were used to test the efficiency of the signal of tissue proliferation of fibroblasts:

1. Therapy for acne ("acne disease").
2. Therapy of age-related skin changes.

In both models, during the study, the following were carried out:

- initial examination of the patient;
- a course of therapy, which included both a specific and a systemic component;
- in some cases - repeated courses of therapy;
- secondary examinations of the patient, including specific, for the selected model, assessing the state of his skin.

Initial patient evaluation in both studies included:

1. Primary diagnosis using the ART method.
2. Clinical examination.
3. Assessment of the severity of the skin manifestations of the consequences of acne.

Diagnostics using the ART method at the stage of primary examination, it was carried out according to the algorithm recommended for doctors [5]. In all patients, the following groups of system indicators were tested without fail:

- geopathogenic load test (Silicea D60);
- test for electromagnetic fields (Phosphorus D60);
- test for radioactivity (Glob. D1000);
- test to determine the level of psycho-emotional stress;
- testing of indicators of metabolic disorders of heavy metals (chakras, according to Schimmel);
- bacterial test (Tetracyclinum D30);
- test for viral burden (Interferon D30);
- tests for burdening with helminths;
- test for the presence of endocrine disorders. In addition, it was carried out:
- testing the biological index (BI) and determining the optimal biological index;
- testing of adaptation reserves (RA) and determination of optimal adaptation reserves;
- determination of violations of macro- and microelements using 17 test indicators of violations of elemental metabolism;
- testing of test indicators of organs and body systems involved in the systemic pathological process (organopreparations);
- testing of test indicators of nosologies and pathogens (nosodes).

Further, all patients underwent a constitutional consistency test for therapeutic use of a preparation of potentiated fibroblasts or a potentiated sum of the fibroblast death signal. For the test, the KMX marker [9] was used as a filter, the sum of the signals of the nodal and terminal BAPs located on the main chiroglyph lines of the patient's palms, written off using an electronic probe, in accordance with the approved BRT procedure [5].

Clinical examination of patients included:

- medical history and family history;

- general examination and clinical and morphological assessment of the skin condition;
- general clinical analysis of blood and urine;
- ultrasound of internal organs;
- biochemical blood tests (ALAT, ASAT, bilirubin, alkaline phosphatase, cholesterol, glucose);

- blood tests for prolactin, testosterone, FSH, LH, estradiol.

The initial course of therapy in both studies consisted of:
- specific therapy aimed at eliminating the poorly treated process of damage to the connective tissue of the face;
- nonspecific therapy aimed at improving the general condition of the patient.

Specific therapy in both studies

For specific therapy, in both studies, in all cases, an individual preparation was used, which is a potentiated signal of the sum of fibroblast death when the ART condition is fulfilled:

KMX ↓ + ∑Pot Rev. fibroblasts ↑.

Patients used the drug of the potentiated amount of fibroblast death in an individually selected potency (the potency of the drug ranged from 5.9-5.6) for 30 days.

Nonspecific therapy in both studies

For nonspecific therapy in both studies, the patient's blood autonosode was used in all cases, aimed at his compactified diagnosis [7].

Re-examination

At the second appointment a month after the start of therapy, a second ART examination was carried out, as well as re-assessment of the degree of skin defects disorders and assessment of changes in skin manifestations after therapy. The condition of the patient's skin was compared during the initial and repeated examination.

Model: acne therapy.

The study involved 19 women aged 18 to 30 years (8 people from 19 to 26 years old with pronounced consequences of acne).

Assessment of the severity of skin manifestations in acne

To assess the degree gravity For acne, the classification proposed by the American Academy of Dermatology was used, where four degrees of disorders are distinguished (mild, moderate, severe, very severe). For a quantitative and qualitative assessment of the severity of the disease, taking into accountsquares lesions used a 0 to 8 scale according to the method of CH Cook et al. (1979) modified by BS Allen and JG Smith.

- the skin is not flawlessly clean, but it is noticeable only at close range - 0 points,
- with an area ¼ or less <1/2 of the total surface of the face - 1–2 points,
- about 1/2 of the entire surface of the face (a little more or a little less) - 3-4 points,
- more than ½, but less than ¾ of the entire area of the face - 5-6 points,
- more than ¾, but less than the entire area of the face - 6-7 points,
- amazed all face - 8 points.

The assessment of the skin condition prior to therapy is given in table. one.

Table 1

Assessment of skin condition prior to therapy

Severity facial skin lesions	Missing	Easy	Average	Heavy	Very heavy
Number of patients with this degree facial skin lesions	2	6	eight	3	0
Affected area face surfaces (in conditional points)	0 points	1-4 points	5-6 points	6-7 points	8 points
Number of patients with this area facial skin lesions	2	6	eight	3	0

Additionally, we note the following anamnestic data:

- 8 patients had tertiary post-inflammatory changes in the skin of the face as a result of postponed constitutional juvenile acne, classification by K.N. Suvorova and N.V. Kotova (1997) on a scale of 5–6 points in clinical assessment (moderate and severe on ¾ of the face surface) with pronounced red spots post-acne, anamnesis from the onset of the disease - from 9 months to 2 years;
- 3 patients had pronounced tertiary changes in the facial skin with atrophic and small keloid scars (severe constitutional juvenile acne, grade 6-7 points, more than ¾ of the surface, disease duration more than 3 years;
- 6 patients with a mild recent lesion of 2–3 points, less than ½ of the face surface, anamnesis from the onset of the disease for 6 months.

Research results

The results of therapy after the first course of treatment (30 days) are shown in table. 2.

table 2

Assessment of the skin condition after a month of therapy (acne model)

Severity facial skin lesions	Missing	Easy	Average	Heavy	Very heavy
Number of patients with this degree facial skin lesions	3	eleven	five	0	0
Affected area face surfaces (in conditional points)	0 points	1-4 points	5-6 points	6-7 points	8 points
Number of patients with this area facial skin lesions	3	eleven	five	0	0

When visually assessing the condition of the facial skin of patients after the first course of therapy, its surface had minimal consequences from acne. The skin in all cases is less bumpy, the surface is smoother, without bluish and bright red spots, normal color.

ART results showed stable good systemic indicators. The results of therapy (31–61 days) after the second course are shown in table. 3.

Table 3

Assessment of skin condition after 2 months of therapy (acne model)

Severity facial skin lesions	Missing	Easy	Average	Heavy	Very heavy
Number of patients with this degree facial skin lesions	eleven	five	3	0	0
Affected area face surfaces (in conditional points)	0 points	1-4 points	5-6 points	6-7 points	8 points
Number of patients with this area facial skin lesions	eleven	five	3	0	0

After 2 months of therapy, the skin condition in all patients had a normal color, a smooth surface, no depressions and no deep traces of acne. If there were single new pustular formations (2 people), skin regeneration occurred fairly quickly without the absence of previous complications.

Model: therapy of age-related skin changes

The study involved 12 women aged 39 to 68 years (of them 4 people with more than 8-10 years old, chronic cicatricial changes in the skin of a youthful acne).

Table 4

Assessment of skin condition prior to therapy

Severity facial skin lesions	Missing	Easy	Average	Heavy	Very heavy
Number of patients with this degree facial skin lesions	6	2	4	0	0
Affected area face surfaces (in conditional points)	0 points	1-4 points	5-6 points	6-7 points	8 points
Number of patients with a given area facial skin lesions	6	2	4	0	0

Assessment of the degree of skin changes with aging

The degree of assessment of the lesion of the skin of the face with age-related changes has a number of features, these are the subjective feelings of the patient himself in assessing his appearance and correlating it with the passport age, the assessment of a person's appearance by his environment and loved ones and the objective vision of the doctor.

Table 5

Assessment of the skin condition after a month of therapy

Severity facial skin lesions	Missing	Easy	Average	Heavy	Very heavy
Number of patients with this degree facial skin lesions	nine	3	0	0	0
Affected area face surfaces (in conditional points)	0 points	1-4 points	5-6 points	6-7 points	8 points
Number of patients with a given area facial skin lesions	nine	3	0	0	0

A month after taking the drug, all patients noted:

- a pronounced improvement in the condition of the skin;
- the relatives around them noticed an improvement in their appearance, although all patients in this period of time did not use any cosmetic procedures to improve their skin condition;

- according to an objective assessment, all those who used the drug had an alignment of the skin of the face, a decrease in fine wrinkles, a clearly noticeable alignment of cicatricial changes in patients with chronic cicatricial skin lesions (4 pers.). ART results showed stable good systemic indicators.

Table 6

Assessment of skin condition after 2 months of therapy

Severity facial skin lesions	Missing	Easy	Average	Heavy	Very heavy
Number of patients with this degree facial skin lesions	10	2	0	0	0
Affected area face surface (in conditional points)	0 points	1-4 points	5-6 points	6-7 points	8 points
Number of patients with given area facial skin lesions	10	2	0	0	0

Discussion

The above results show that the problem of restoring both the functions and the structure of the connective tissue in the body is solvable by using special control signals, in this case the control signals of fibroblast death obtained by the authors. At the same time, additional study of a number of issues is required:

1. To what extent is the signal used is specific, i.e. with what therapeutic efficacy, it could be replaced by a different control signal (CS), which would not be derived from the signal of fibroblast death, but would compensate for the same group of test indicators.

2. Using the CA of cell culture death (death of fibroblasts) in order to solve gerontological problems require a much more thorough objectification of the criteria for the action of this signal using special experimental techniques supported by appropriate equipment.

Conclusions:

1. Application of US of fibroblast death promotes skin restoration during therapy acne and age-related changes.

2. The effectiveness of therapy using control signals of tissue proliferation fibroblasts on models of acne and age-related skin changes is comparable to the results

cell therapy and significantly exceeds the effectiveness of traditional methods currently used in cosmetology.

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