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Autologous dermal fibroblasts for the correction of age-related skin changes (SPRS-therapy®). Results of 2-year clinical trials and post-marketing clinical studies

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Abstract

One of the innovative methods to correct the age-related skin changes is the use of cultured fibroblasts derived from the patient's own skin. The technology called SPRS-therapy® (Service for Personal Regeneration of Skin) has been officially approved in 2011 in Russia (developed by the public biotech company Human Stem Cells Institute (HSCI), Moscow).

***The results of two-year clinical trials* with the application of SPRS-therapy to correct the age-related skin defects demonstrated, that after the intradermal transplantation (injections), cultivated autoDF (autologous dermal fibroblasts) fully integrated into the dermis and their biosynthetic activity maintained at least for 12 months. As a result, the increase of collagen content and skin hydration was observed as well as the increased thickness of the dermis. The abovementioned changes resulted in the increase of skin tension, elasticity, and thickness and in the decrease of an amount of wrinkles and their depth.**

***The results of post-marketing studies* conducted by HSCI after the first year of the application of technology in clinics confirmed the safety and clinical efficacy of SPRS-therapy for correction of skin aging changes.**

On the aesthetic medicine market, SPRS-therapy was introduced by HSCI in January 2011. Today the SPRS-therapy service is primarily offered through the leading dermatology and cosmetics clinics of Moscow and cities across Russia and CIS (>40). Currently, the total number of patients that received the procedure is more than 750, with more than 60% of those that have returned for the second and/or additional skin treatments (included in other areas besides the face).

***SPRS-therapy complex, developed for correction of patient's skin changes and aging prophylaxis*, includes also SPRS-diagnostics service (the evaluation of morpho-functional properties of patient's skin fibroblasts called Skin Passport®), which allows to build an individual SPRS-therapy program as well to provide recommendations on other instrumental and cosmetological procedures for the patient. An individual cryogenic bank of skin fibroblasts is created for each patient allowing to repeat the procedure on a regular basis without additional skin sampling.**

Introduction

Aging skin, as well as the organism aging in general, represents a complex biological process which is influenced by many factors including genetic, epigenetic, and environmental ones. It has been demonstrated that processes developing in the skin during aging are based on fundamental changes connected with the main cellular population of the derma and fibroblasts, namely, with their amount, morphology, proliferative potential, and functional activity[1-4]. In the skin, fibroblasts are responsible for the whole range of various and sophisticated

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functions. They not only maintain homeostasis of the dermal intercellular matrix supporting its remodeling and renewing (due to degradation of "used" dermal components and synthesis of the new ones – collagen, elastin, and the main substance), but also play a significant role in maintaining the physiological status of the other skin layers representing a key chain in the skin metabolism[1].

When age increases, the amount of dermal fibroblasts is decreased, as well as their ability to synthesize biomolecules, and the balance between synthesis and degradation of components of the intercellular dermal matrix is destroyed. Thus, the study of Varani et al[2] has shown that in old people the total amount of dermal fibroblasts is averagely less by 35% as compared with young people. According to the data of Fisher et al[4], the collagen production in the skin of old people is decreased by 75% on average. The natural consequences of such a decrease include thinning of the skin, the loss of the skin's flexibility and elasticity, and wrinkle formation.

Thus, the age-related skin changes are accompanied by decreasing the dermal population of fibroblasts as well as their proliferative and biosynthetic abilities, which results in deterioration of quantitative and qualitative characteristics of the dermal intercellular matrix.

Currently, in the practice of aesthetic medicine, a large arsenal of methods is used such as peels, fractional photothermolysis, radio wave therapy, dermabrasion, etc., with the main purpose of the application being stimulation of the functional activity of fibroblasts. A special place in this series is the method relating to regenerative medicine – the use of cultured fibroblasts derived from the skin of the patients themselves to correct their age-related changes. The peculiarity of this technology is that it allows you to replenish age-diminished with the population of resident fibroblasts of young and functionally active cells, grown in a special laboratory environment that allows for the unique biological mechanisms effectively to correct age-related skin defects.

As early as in 1995 (Boss Jr., et al), it was shown that intradermal administration of autologous dermal fibroblasts (autoDF) promoted the effective correction of aging skin changes[5]. Cultured dermal fibroblasts actively synthesized collagen and other components of extracellular matrix *in vitro*, and after dermal transplantation, such synthetic activity remained. Later (2003-2008) the company Fibrocell Science, previously known as Isolagen, (USA) conducted multiple randomized, blinded, placebo-controlled clinical studies for the treatment of facial wrinkles. And in July 2011, this technology was used in the USA as technology LAVIV™ (azticel-T)[6].

Since July 2010, the use of the technology of intradermal administration of autologous dermal fibroblasts has been officially approved in Russia[7]. The technology is termed the SPRS (Service for Personal Regeneration of Skin) therapy (OJSC "Human Stem Cells Institute").

SPRS-therapy represents a set of personalized diagnostics and treatment procedures which help to improve skin appearance based on the innovative technology of applying autoDF to correct skin damage due to aging and other structural changes.

The clinical results of the SPRS-therapy

The Human Stem Cells Institute in cooperation with the Central Research Institute of Dental and Maxillofacial Surgery (Moscow) and with participation of a number of Moscow cosmetic clinics conducted two-year clinical trials (non-randomized, prospective, open-label, single-group study with comparison to baseline facial skin status) with the application of autoDF to correct the age-related skin defects in order to obtain the data on duration of clinical effect, the persistence of biosynthetic activity of transplanted fibroblasts, and mechanisms of qualitative and quantitative changes occurring in human skin after injections of fibroblasts.

Patients. The study enrolled 17 healthy subjects (4 men, 13 women) at the age of 45-65 years (the mean age – 53 years) with mild to moderate signs of aging facial changes (wrinkles, reduced skin turgor), who did not receive other treatments to correct aging facial changes in the study period (were eligible for inclusion).

Ethics. The clinical studies were carried out in accordance with the medical technology approved by Russian Healthcare Regulation Authority (Roszdravnadzor)[7] and by the decision of the Ethics Committee and Academic Council of the Central Research Institute of Dental and Maxillofacial Surgery (#4/276 from 14.04.2010). All patients signed the informed consent.

SPRS-product (autoDF) represents a suspension of fibroblasts which are derived from skin (4 mm) taken from behind the patient's ear (an area which

is exposed to harmful UV-rays to the least extent), in concentration $15x10^6$ cells/ml of saline solution for injections.

Characterization of autoDF

Immunophenotype. The immunophenotypic analysis of dermal fibroblasts cultures showed high level of collagen (type I, III), elastin and vimentin expression, revealed markers (CD73+, CD90+ and CD105+) which confirmed mesenchymal origin of used cells and lack of hemopoietic cell (CD34-, CD45-, and epithelial cell (cytokeratins 14, 15, 16, 19) markers. Cytoskeletal analysis revealed the morphology of actin filaments which was typical for mesenchymal cells.

Stability of genetic apparatus. The cytogenetic analysis of cultured dermal fibroblasts at 4-10 passages which was based on the method of differentially stained chromosomes showed that their 3 chromosomal apparatus remained stable in such conditions of cell isolation and culture[8].

The treatment course. The treatment course consists of 2 procedures with injections of autoDF per $60x10^6$ cells in concentration $15x10^6$ cells/ml of saline solution for injections, with a 4-6 week interval. Cell material is administered as intradermal linear retrograde injections in the papillary dermal layer, with needles 30-32G 13 mm long. One hour before the procedure, anesthetic cream EMLA is applied to the skin.

The results of clinical studies. The results of the two-year clinical and morphological studies demonstrated that during the complete period of follow-up, statistically significant skin improvement was observed as compared with the initial level: the increase of thickness of the dermis (on average by 63%), the decrease of an amount of wrinkles and their depth, and the increase of skin tension and elasticity[8,9].

The improvement of facial skin state, including the increase of skin elasticity, the decrease of skin relief, and the improvement of skin color and facial contours, was noticed by both patients and physician-investigators 10-14 days after finishing the course of cell therapy. The clinical effect constantly (for 6-8 months) increased and was maintained at least for 2 years[8,9].

The histological study of skin biopsy materials. The histological analysis of patient's skin biopsy materials showed: in one month after administration, autoDF were visualized in derma as small groups, without signs of mitosis; newly synthesized elements of the intercellular matrix, newly synthesized collagen fibers, increased dermal volume and hydration were observed[8,9]. In injection sites, cultured autoDF remained at least for 12 months. Hereby, the prolonged synthetic activity was observed which was manifested as a synthesis of young (argyrophil) collagen fibers shown as thin crimped black threads within fibroblast groups. It resulted in a progressive increase of dermal thickness which was shown as changes of the mean distance between cutaneous appendages (Figure 1).

The immunohistochemical skin analysis after SPRS-therapy showed that transplanted cells did not proliferate and have no pathological differentiation (i.e., to myofibroblasts) which was demonstrated with antibodies to Ki-67 and smooth muscle actin (α-SMA), which, in its turn, neutralized the minimal theoretical risk of excessive proliferation of transplanted cells or fibrous tissue formation. All preparations did not reveal an excessive number of phagocytes in derma, their number in injection sites and surrounding tissues did not significantly differ[8,9].

There were positive dynamic skin changes after application of autoDF on the histological level which is fully consistent with the clinical picture. Improvement of facial skin – increased firmness, reduced relief, improved facial color and contours – was observed by patients within 10-14 days after the course of cell therapy. The effect was progressive. Thus, if 88% of patients evaluated the clinical

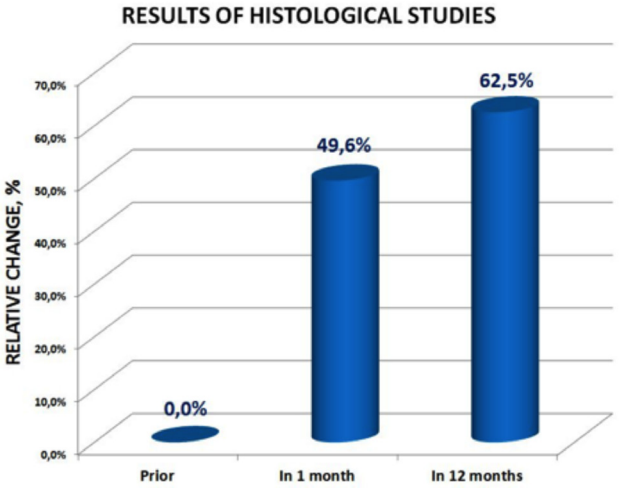

Figure 1. Relative change of skin thickness after intradermal therapy with autoDF (according to the results of histological studies).

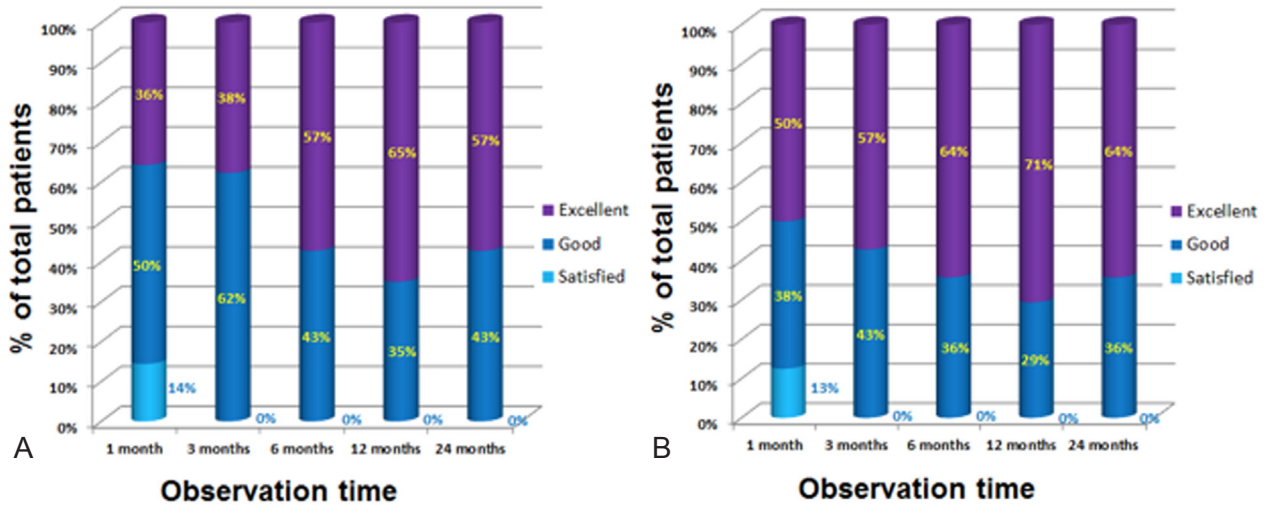

Figure 2. Visual evaluation of facial skin (on 5-score scale): evaluation by a study doctor ***(A)***; evaluation by patients ***(B)***.

results as "good" and "excellent" in 1 month; then in 3, 6 and 12 months – evaluations became 100%. The assessment by the study doctor almost coincided with patient's assessment: in 1 month, the doctor assessed the results as "good" and "excellent" in 86% of patients; in 3, 6 and 12 months were assessed almost 100% – in all study participants. Any systemic or serious local adverse reactions were not reported (excluding bruising and swelling resolving in several days).

The obtained results allowed one to conclude that after the intradermal transplantation, cultivated autoDF were fully integrated into the dermis and their biosynthetic activity was maintained at least for 12 months. As a result, the increase of collagen content and skin hydration was observed as well as the increased thickness of the dermis. The above-mentioned changes resulted in the increase of skin tension, elasticity, and thickness and in the decrease of an amount of wrinkles and their depth (Figure 3).

In the application of SPRS-therapy, the patient invariably raises the question how often he can repeat of SPRS-therapy and what kind of cosmetic methods may be used with regards to their exposure on skin layers to achieve stable aesthetic results without impaired dermal microstructure. In this regard, we have developed SPRS-diagnostics ("Skin Passport®") that allows, from our point of view, to answer these questions by defining the regenerative and proliferative potentials of a population of dermal fibroblasts of patients skin.

SPRS-diagnostics represents the evaluation of the morpho-functional properties of patient's skin fibroblasts by means of a proprietary method of clonal analysis[10] which allows to determine:

– Efficacy of fibroblast colony formation (ECO-f) in primary culture derived from patient's skin. On the basis of obtained data, the conclusion is made about *regenerative* potential of patient's skin fibroblast population;
– Ratio of colony proportions depending on the mitotic activity of fibroblasts forming such colonies. On the basis of obtained data, the conclusion is made about *proliferative potential* (PP) of patient's skin fibroblast population.

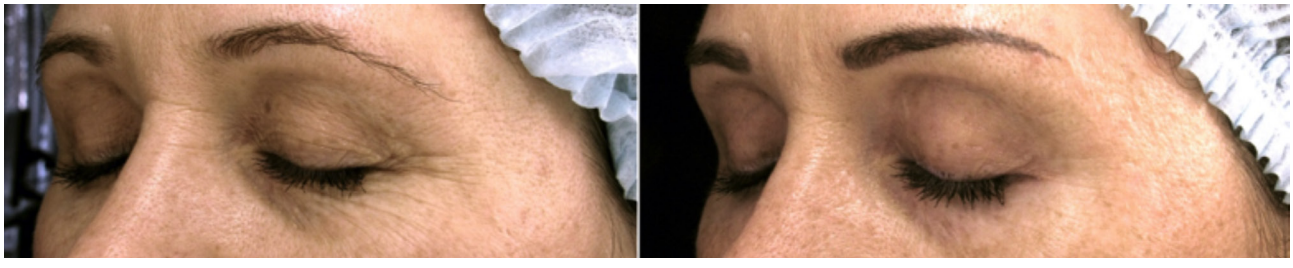

Figure 3. Photo of Patient K. 54, *before* and 12 months *after* administration of autoDF.

It is known that dermal regeneration in adult organism occurs due to the function of stem/progenitor stromal cells originating differentiating/differentiated cells[1,10]. Such progenitor stromal cells found in tissue are determinative for *regenerative potential of* connective tissue – ability of tissue cell population to fully recover its structural components instead of lost ones (as a result of ageing or damage of various genesis). The rate of the process can be evaluated on the ability of such cells to proliferation – *proliferative potential* – ability of cells to multiple fissions to preserve functionally active cell population in tissue. The higher is regenerative and proliferative potential of cells forming derma, the more active is tissue regeneration process and vice versa [11]. The above-mentioned parameters supplement each other in general terms of tissue regeneration efficacy and define the possibility of tissue regeneration – presence and number of "active sites" of regeneration (regenerative potential), as well as kinetic features of the process (proliferative potential).

Comparing the results of ECO-f and PI in patient's skin fibroblasts with reference (normal for the group) values, we made the conclusion about the morpho-functional state of the patient's cell population and its ability for dermal recovery.

On the basis of the results of SPRS-diagnostics, an individual program (*SPRS-program*) is developed for correction of patient's skin changes and aging prophylaxis. SPRS-program represents a complex of recommendations on a number of SPRS-therapy procedures, their terms and cosmetic methods with regards to their exposure on skin layers to achieve stable aesthetic results without impaired dermal microstructure.

From our point of view, determining the value of the regenerative and proliferative potential of dermal fibroblasts is of great significance since it can help physicians-cosmetologists not only to create the optimal skin care program, but also to predict the extent of clinical effect for any patient after application of the intradermal procedure. For example, if these values are low, any cosmetic procedures should be conducted with a certain caution so that not to deplete a small pool of fibroblast precursor cells. Such patients need the course of SPRS therapy to replenish the pool of functionally active dermal fibroblasts before conducting any procedures influencing the skin.

The results of the introduction on the market of aesthetic medicine

On the aesthetic medicine market, SPRS - therapy displayed as a public biotech company, Institute of Human Stem Cells (HSCI) in January 2011. The service is available through skin care clinics (cooperation with clinics in 16 cities of Russia and CIS countries) and to date, the total number of patients is more than 700, with more than 60% of patients having repeated treatments (two or more times) for the treatment of skin in other areas.

Indications to conduction of SPRS-therapy:

- Aging changes of facial and body skin (skin thinning, reduced skin firmness and elasticity, present wrinkles);
- Skin rehabilitation after peelings and plastic surgeries;
- Post-acne scars.

The results of post-marketing studies conducted after the first year of clinical application of this technology confirmed the safety and clinical efficacy of SPRS-therapy for correction of skin aging changes.

Results of post-marketing clinical studies

The post-marketing clinical studies which were carried out by HSCI from January 2011 to January 2012 to examine the safety and clinical efficacy of SPRS-product in one hour after the therapy showed that all (!) studied subjects were satisfied with the clinical results. 93 patients took part in the study, among them 14% – men, 86% – women, the average age of patients – 48 years. So 100% of patients were satisfied with the clinical results (Table 1)[12].

Table 1. Results of post-marketing clinical studies of SPRS-therapy in Russia (1 year of observation).

Test value	Results
Total number of clinics participated	8
Number of patients who have SPRS therapy (skin of face)	93
Number of surveyed patients	93
Number of complications	0
Number of reported adverse effects	21 (22.6%)
Number of adverse events	0
Number of patients not satisfied with results of therapy	0
Number of doctors not satisfied with results of therapy	0
Reported signs of efficacy	Increased skin firmness, elasticity, thickness; decreased number and deepness of wrinkles; lifting effect; correction of skin color and face oval

Table 2. Results of post-marketing clinical studies of SPRS-therapy in Russia (1 year of observation).

Indicator of efficiency	Estimation (number of patients, %)
Increase of skin thickness and decrease of wrinkles depth	100
Increase of skin firmness and elasticity, lifting effect	87
Decrease in the number of wrinkles	73
Complications, negative effects	0

All patients observed the increased skin thickness and decreased wrinkle deepness, 87% – increased skin firmness, elasticity and lifting effect, 73% – decreased wrinkles (Table 2). No complications have been reported, adverse events – hemorrhages and short-term swelling (which were common for the technology and resolved within 5-7 days) – were observed in 22.6% of patients, papules up to 2 mm (resolving within 1-2 months) were observed in 3.6% of patients. Over one-third of patients who took dermal autoDF for correction of a skin area, applied to the clinic again for therapy of other skin areas (neck, decollete zone, hands)[12].

Conclusions

One-year post-marketing studies confirmed: the technology was safe and clinically effective.

It should also be noted that SPRS (Service for Personal Regeneration of Skin) allows you to create an individual cryogenic bank of skin fibroblasts for each patient: some portion of cultured cells is preserved in the cryogenic bank, where they can be kept in liquid nitrogen for an indefinite period of time. Cells preserved in such a way can be used for the production of SPRS-product for patient's lifetime.

Good Publication Practice

All phases of clinical trials were conducted according to the Declaration of Helsinki of the World Medical Association ''Recommendations guiding physicians in biomedical research involving human subjects'' (1964, 2000), ''Rules of Good Clinical Practice in the Russian Federation'' OST 42-511-99, ICH GCP rules, and valid regulatory requirements.

Conflict of Interests:

The Authors declare that they have no conflict of interests.

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