# Clinical-instrumental and morphological evaluation of the effect of autologous dermal fibroblasts administration

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## Abstract

Basic molecular mechanisms, associated with the main cell population of the dermis – fibroblasts – are the basis of skin aging. The number of functionally active fibroblasts in the skin and their biosynthetic activity decreases with age, thus enhancement of their cell density with synthetically active cells is accepted as a one of the most effective methods. The objective of the present study was to evaluate the safety and effectiveness of intradermal administration of autologous dermal fibroblasts in a year after treatment of 17 patients, aged 45–65 years. Results obtained with modern instrumental skin diagnostic methods (vacuum cutometry, optical profilometry, VISIA photometric analysis, etc.) demonstrate the safety and clinical effectiveness of dermal autofibroblast therapy: after transplantation, cultured autofibroblasts keep their biosynthetic activity and produce extracellular matrix for at least 12 months. As a result, remodelling of the dermis microstructures is observed, accompanied by a progressive increase of collagen content and thickness of the dermis (up to 62.5  $\pm$ 6.7% in 12 months). This is clinically expressed by increase of skin elasticity (24.0  $\pm$ 4.3% in periorbital area) and thickness of the skin, and by decrease in the number and depth of wrinkles (46  $\pm$ 7% by the end of observation period). Copyright © 2014 John Wiley & Sons, Ltd.

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## 1. Introduction

Skin aging is based on fundamental molecular mechanisms which are associated with the primary dermal cell population – fibroblasts. The main function of fibroblasts is to produce, organize and renew intercellular dermal matrix (Fisher *et al.*, 2008; Sorrell and Caplan, 2009). It is known that dermal fibroblasts, the heterogeneous cell population, which consists of all fibroblastic programmed differentiation from multipotent mesenchymal stromal cell, progenitor cells and differentiated fibroblasts up to finally differentiated fibrocytes, are the main effectors in skin physiology (Sorrell and Caplan, 2009). They control composition and structure of intercellular matrix via feedback-regulated synthesis of collagen, elastin and cytosol, as well as involvement in degradation of the components (Zhukova *et al.*, 2009).

With age, the number of functionally active fibroblasts is decreased in the skin, the balance between synthesis and degradation processes of intercellular matrix is disrupted, their biosynthetic activity is reduced and the collagen content (the main structural dermal component) is decreased (Varani *et al.*, 2006; Fisher *et al.*, 2008; Sorrell and Caplan, 2009). It is shown that collagen production in skin of elderly persons (aged over 80 years) in comparison with the young (aged 18–29 years) is reduced on average by 75%, and the total number of fibroblasts is decreased by an average of 35% (Fisher *et al.*, 2002). Such processes manifest as decreased skin thickness, decreased elasticity and wrinkle formation.

Boss *et al.* showed, in 2000, that intradermal administration of autologous dermal fibroblasts (autoDF)

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promoted the effective correction of changes in aging skin. Cultured dermal fibroblasts actively synthesized collagen and other components of extracellular matrix (ECM) *in vitro* and, after dermal transplantation, such synthetic activity remained.

Currently, aesthetic medicine in a few countries has officially approved the use of autoDF for correction of changes in aging skin. Since July 2010, the technology is officially approved in Russia [SPRS-therapy; OJSC 'Human Stem Cells Institute' (HSCI), Moscow, Russia] (Zorin *et al.*, 2010), and since July 2011, in the USA [LaViv<sup>TM</sup> (azficel-T; Fibrocell Science, Inc. (former Isolagen Inc.), Exton, PA, USA) (Fibrocell Science Inc, 2011).

The accumulated experimental and clinical materials conclusively prove safety and clinical efficacy of autoDF for correction of skin defects, but meanwhile, it should be admitted that some problems should be reviewed. In particular, such aspects as the duration of biosynthetic activity of transplanted autoDF and the dynamics of dermal changes after injection, are insufficiently disclosed in scientific literature.

The aim of the present clinical study was therefore to obtain objective data about the duration of synthetic activity of transplanted autoDF and nature of changes that occur in human skin after administration of the cells, based on a complex approach encompassing morphological, laboratory and clinical instrumental methods.

## 2. Materials and methods

#### 2.1. Study design

The design of the clinical study was a non-randomized, prospective, open-label, single-group study using a comparison with baseline facial skin status (Figure 1).

## 2.2. Patients

The study enrolled 17 healthy subjects (4 men, 13 women) in the age range of 45–65 years (mean age 53 years) with mild to moderate signs of aging facial changes

(wrinkles, reduced skin turgor). All patients included did not receive other treatments to correct age-related facial changes during the study period. The exclusion criteria for participation included pregnancy or lactation, history, evidence of both of chronic facial skin disease and disorders, recent use of systemic or topical corticosteroids, oncological diseases, autoimmune diseases of the connective tissue, human immunodeficiency virus (HIV), hepatitis B or C, as well as patients that had undergone procedures of skin rejuvenation 6 months before the study.

#### 2.3. Ethics

The clinical studies were carried out in accordance with the medical technology approved by the Russian Healthcare Regulation Authority (Roszdravnadzor) (Zorin *et al.*, 2010) and 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 form.

# 2.4. Derivation and characterization of autoDF cultures

Fibroblasts were obtained by skin punch biopsy (4 mm) retroauricular (from the area least subjected to solar UV irradiation damage) in each individual patient under local anaesthesia with 2% lidocaine (Egis Pharmaceuticals, Budapest, Hungary). The primary cells were isolated via enzyme digestion following by expansion of fibroblasts in GMP laboratory for 6–8 weeks in Dulbecco's modified Eagle's Medium (DMEM) medium conditioned with 10% fetal bovine serum (FBS) (Hyclone; Thermo Scientific, Waltham, MA USA). No special supplements or growth factors were added during cultivation.

#### 2.5. Administration of cell product

One hour before the procedure, anaesthetic cream EMLA (AstraZeneca GmbH, Wedel, Germany) was applied to facial skin. The autoDF suspension was administered to



Figure 1. The study design scheme. Treatment = two injection sessions performed at 1-month interval; Examinations = examinations of patients which include instrumental and histological tests, and clinical analysis made before treatment and in 1, 3, 6 and 12 months after the treatment

patients twice in a 1-month interval with  $60 \times 10^6$  cells per session (and a concentration of  $15 \times 10^6$  cells/ml in saline solution) using a linear intradermal injection technique. The autoDF were injected with a 30-gauge, 13 mm needle to the papillary dermis, 0.05 ml per injection to 2 ml (about 40 injections in total) per each side of a face according to the scheme (see Supporting Information, Figure S1).

Concomitantly, the cell material was injected to the skin behind the patient's ears for subsequent biopsy and histological testing (one for the test and another as a control).

#### 2.6. Laboratory study methods

#### 2.6.1. Safety measures

The cell material was tested to exclude viral, bacterial and fungal agents using polymerase chain reaction (PCR) method and standard bacteriological analysis in a certified diagnostic centre. The final cell products were thoroughly washed for 24 h before harvesting to remove any traces of growth media supplements.

#### 2.6.2. Immunophenotypic analysis

Specific markers for each type of cells were quantified by flow cytometry. A single-cell suspension ( $10^6$  cells) was fixed with 4% paraformaldehyde (Cat. No: P6148; Sigma-Aldrich Corp., St. Louise, MO, USA) solution and incubated in 100 µl of phosphate buffered saline with fluorescencetagged primary antibodies (CD34PE, CD45FITC, CD73PE, CD90APC, CD271PE, pan-cytokeratin PE 14, 15, 16, 19, Cat. Nos 550761, 555482, 550257, 559869, 557196, 550953; BD Pharmingen, Franklin Lakes, NJ, USA) and CD105 Alexa 488 (Cat. No. MHCD10520; Invitrogen, Carlsbad, CA, USA) in 1:100 dilution at 37°C for 30 min in dark.

Other markers were stained with primary mouse monoclonal Col1 (Cat. No. 2150–0001; AbD Serotec, Oxford, UK), vimentin (Cat. No. M0725; DAKO, Glostrup, Denmark), polyclonal rabbit Col3, elastin (Cat. Nos 2150-0100, 4060-1060; AbD Serotec, Oxford, UK) unconjugated antibodies, and secondary Alexa FluorR488 Goat Anti-Mouse IgG (H+L)/Alexa FluorR488 Chicken Anti-Rabbit IgG (H+L) antibodies (Cat. Nos A-11029/A-21441; Invitrogen, Carlsbad, CA, USA). In all experiments corresponding isotype control antibodies were used. The percentage of positive cells was evaluated based on the fluorescence. In total, 50 000 events were acquired using a FACSCanto<sup>™</sup> II flow cytometer and analyzed using FACSDiva<sup>™</sup> Becton Dickinson software (BD Biosciences, http://www.bdbiosciences.com).

## 2.6.3. Efficacy of colony formation (ECF-f)

The ECF-f was determined with original clonal analysis. Skin fibroblasts of the second passage were cultured in three Petri dishes 100 mm in diameter with cultural medium DMEM supplemented with FBS Defined (Hyclone; Thermo Scientific) and culture density 100 cells per dish. Cells were cultured in CO<sub>2</sub> incubator in saturated humidity conditions at +37°C in atmosphere 5% CO<sub>2</sub> for 14 days. Petri dishes with formed colonies were then washed, fixed and stained with KaryoMAX® Giemsa Stain (Gibco/Life Technologies Inc., Grand Island, NY, USA). Formed colonies were scanned with ChemiDoc<sup>TM</sup> XRS Universalhood II system (BioRad Laboratories Inc., Hercules, CA, USA) and processed with colonies were counted with ImageJ software ver.1.46 (http://imagej.nih.gov/ij/). EFC-f was considered as the percent ratio of formed colonies with number of cells >20 to total number of explanted cells.

#### 2.7. Instrumental study methods

All measurements were made in six areas symmetrical to the face axis: periorbital, buccal and perioral.

#### 2.7.1. Vacuum cutometry

Changes of skin mechanic properties, in particular, its elasticity before and after administration of autoDF, were evaluated with Cutometer RMPA 580 (Courage+Khazaka electronic GmbH, Cologne, Germany).

In the studies, 'biological elasticity' (parameter R2; Spitale *et al.*, 2012) – the ratio between residual deformation and maximal range of skin deformation – was used to evaluate skin elasticity (Draaijers *et al.*, 2004).

The following parameters were used for the measurements: constant negative pressure, vacuum value of 450 mBar, sensor aperture 2 mm, suction time (ontime) 1.5 s, relaxation time (off-time) 1.3 s, 10 repeated measurement cycles, 0 s rest time, temperature  $22 \pm 1^{\circ}$ C and relative humidity 50  $\pm 10\%$ . Before measurements, all patients were kept in preconditioned room for 10 min with relaxed facial muscles.

#### 2.7.2. Photometric analysis of facial skin status

Dynamics of skin texture changes and number of wrinkles was evaluated with photometric system VISIA<sup>TM</sup> Facial Complexion Analysis System, Ver.4.0c (Canfield Scientific Inc., Fairfield, NJ, USA) and Visia 5.2 software (Spitale *et al.*, 2012).

To make measurements the instrument camera was set to the superfine image capture mode (400 dpi).

The following parameters were used for photography in the different modes. For UV mode, an aperture of f/4.0, ISO of 100, shutter speed of 1/60 and white balance set to daylight was used. For the cross-polarized mode an aperture of f/8.0, ISO of 50, shutter speed of 1/100, and white balance set to daylight was used. For the visible photography, an aperture of f/5.6, ISO of 100, shutter speed of 1/60 and white balance set to fluorescent was used.

A mask delineating the area of analysis on the face was drawn by the software and adjusted by the camera operator to include the forehead and cheeks and to exclude nose, the eyes and facial hair.

#### 2.7.3. Optical profilometry

The Phaseshift Rapid *In Vivo* Measurement of the Skin (PRIMOS) system was used (GFMesstechnik GmbH, Teltow, Germany) to study dynamics of changes in wrinkle deepness (Jacobi *et al.*, 2004).

The PRIMOS system represented a non-contact, prompt and high-precision method (resolution 0.004 mm) for plotting a three-dimensional (3D) skin model and allowed measurement of wrinkle deepness with a high degree of accuracy. The PRIMOS software, Version 6, allows rapid calculation of wrinkle deepness and dynamic comparison of skin areas tested, combining two data sets measured at different time-points.

To illustrate and measure the dynamic changes in wrinkle deepness, the metric parameters of the baseline measurements of the selected 3D image skin profile were compared with those 1 month and 12 months after autoDF administration by PRIMOS software. The difference between maximal and minimal peaks on the profilogram was used as a parameter to characterize wrinkle deepness.

## 2.7.4. Clinical analysis of facial skin status

The wrinkle area fraction and skin elasticity were measured by objective evaluation using previously validated (Lemperle et al., 2001) 6-score Facial Fold Assessment Scale. In addition, a satisfaction survey of the subjects was conducted on the basis of subjective evaluation according to the guideline for the evaluation of anti-wrinkle effects. In this study a significant improvement was observed in the facial skin wrinkles evaluated by three independent specialists in six areas along a bilateral facial symmetry axis before and after autoDF administration. The assessment scale was a five-point scale from 0 (no wrinkles) to 5 (very deep and evident wrinkles and skin folds). The assessment scale was developed and evaluated by doctors blinded to the treatment received to eliminate any potential for bias based on the injection of autoDF. Photographs were taken of each subject at baseline and at selected times after treatment with the imaging system VISIA. An overall improvement score was computed for each subject and calculated by averaging the improvement across facial areas according to the following scale: 'excellent effect' = 2.0, 'good effect' = 1.0-2.0, 'satisfactory effect' = 0.5-1.0, 'no effect' = 0.0-0.5 and 'negative effect' = -1.

The independent photographic reviewers were blinded to the treatment or no treatment photographs. The patients themselves evaluated the clinical effect with qualitative evaluation system according to the same scale.

#### 2.7.5. Histological study

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Retro-auricular skin biopsies were taken before the administration of autoDF with two or three patients in each control period. After tissue processing, H&E (haematoxylin and eosin) staining was carried out to evaluate morphological changes concerning epidermal thickness and average distance between appendages of skin. Furthermore, Gordon silver impregnation was performed to evaluate the dermal collagen density.

For further characterization of autoDF, as well as confirmation that cells observed in the dermis after transplantation of autoDF are transplanted autoDF, immunohistochemistry was performed on paraffin sections using antibodies against CD45 and vimentin (DAKO).

### 2.7.6. Statistical analysis

Values obtained were expressed as mean  $\pm$  standard deviation (SD). The level of statistical significance was set at 5%. The main statistical calculations were made with IBM SPSS Statistics version 20 software (IBM Corp., New York, NY, USA).

Normal distributions were tested before performing comparisons. The results of instrumental studies in patient groups were evaluated before and after autoDF administration with parametric (Student–Newman–Keuls test, SNK) and non-parametric (Wilcoxon signed-rank test) methods for repeated measures analysis of variance.

The correlation analysis to determine the relationship between non-qualitative parameters have been made with calculation of a Spearman's rank correlation coefficient.

# 3. Results

### 3.1. Patients

All 17 patients participated in clinical trials in the observation period. No adverse events or serious adverse reactions were reported, except for small bruises and swelling (common for intradermal administration) which resolved in several days. All patients were satisfied with the clinical results.

#### 3.2. Immunophenotype of cultured autoDF

Immunophenotype analysis of the dermal autofibroblast cultures confirmed the mesenchymal origin of the cells with a high level (>99%) of collagen type I and III, elastin and vimentin expression; markers were positive for CD73, CD90 and CD105 (mesenchymal stem cell markers) and negative for CD34 and CD45 (haemopoietic cells) as well as cytokeratines 14, 15, 16 and 19 (epithelial cells). Cytoskeletal analysis showed mesenchymal stem cells (MSCs) typical for the morphology of actin filaments.

The analysis of quantitative values of elastin, collagen of type I and III proteins expression in autoDF did not reveal statistically significant relationship with patients' age and sex (p > 0.05).

#### Evaluation of the effect of dermal fibroblast administration

#### 3.3. Evaluation of colony formation efficacy

The ECF-f was found to be in the range 10–65% (mean 45.0 ±9.5). Thus, no statistically significant relationship with patient's age and only minor, statistically insignificant gender dependence were observed (p = 0.065).

#### 3.4. Study of facial skin elasticity

The dynamics of absolute changes concerning skin elasticity after the second intradermal autoDF procedure are shown in Figure 2. The maximal increase of facial skin elasticity was observed in periorbital area after 6 months (24.0  $\pm$ 4.3%) and remained almost at the same level up to the end of observation period. The effect was progressive in all areas tested, reaching the plateau phase after the sixth month. Statistically significant changes in facial skin elasticity were recorded in the periorbital area by 1 month, and in the buccal area in 3 months. The perioral area, despite the tendency to increased elasticity, showed insignificant changes (p > 0.05).

#### 3.5. Photometric complex (Visia) studies

Studies made with photometric complex 'Visia' showed progressive improvement of facial skin texture and relative reduction of wrinkles throughout the observation period (Figure 3), with maximum levels of 29  $\pm$ 5% and 46  $\pm$ 7%, respectively, reached after 1 year.

The Visia photographs (Figure 4) reflected the dynamics of skin texture changes and number of wrinkles based on the case of study patient (patient M, 53). The studies, which were carried out with optic profilometry with the Primos Lite system (GFMesstechnik GmbH) also



Figure 3. Relative change of skin parameters after administration of autoDF (Visia<sup>TM</sup>). \*Statistically significant change over baseline, p < 0.05

confirmed the dynamics and type of decreased wrinkle deepness in individual areas of patients' faces.

The clinical evaluation of patients' facial skin showed a progressive decrease in wrinkle number and deepness in all face areas tested 1 month after autoDF administration (Table 1). The number of patients with maximal scores was close to 100% to at the end of observation period (12 months). Consequently, 'good' and 'excellent' scores for the intensity of clinical effect were detected in 100% of patients by month six after autoDF administration. As expected, the visual analysis of facial skin made by patients showed an ever more optimistic tendency (Table 2).

The histological studies showed intradermal evidence of the administered autoDF, assorted as cell groups without signs of mitotic activity, 1 month after the procedure



Figure 2. Changes in elasticity of skin (mm) in 1, 3, 6 and 12 months after administration of autologous dermal fibroblasts (autoDF) (parameter R2, gross elasticity). \*Statistically reliable change over baseline, p < 0.05



Figure 4. Relative change of skin parameters (A, C change of wrinkle number; B, D change of skin texture) before and after administration of autoDF (Visia<sup>TM</sup>, patients M, 53 years and K, 57 years)

(Figure 5). The great amount of newly synthesized elements of ECM was observed in the area of cultured autoDF injection; in transplantation sites, autoDF were detected throughout the observation period (1, 3, 6 and 12 months) and the presence of young (argyrophil) collagen fibres, shown as thin crimped black threads within fibroblast aggregations, (Figure 6) was reported in all cases.

During the period of observation, cells in fibroblast aggregations intensely expressed vimentin; at later stages vimentin-positive cells were equally distributed in the dermis (see the Supporting Information, Figure S2). The immunohistochemical studies showed that CD45 expression in the cell groups was insignificant, regardless of the time after transplantation (see the Supporting Information, Figure S3). These data confirm the mesenchymal nature of the cells observed.

Table 1. Results of visual assessment of facial skin (6-score Facial Fold Assessment Scale), which was made by three independent study doctors

Skin evaluation, average score	Before injection, <i>n</i> /%	Months after injection of AutoDF			
		1, <i>n</i> /%	3, n/%	6, <i>n</i> /%	12, <i>n</i> /%
0 point 1 point 2 points 3 points 4 points 5 points	0/0 0/0 3/18 10/59 4/24 0/0	0/0 0/0 8/47 9/53 0/0 0/0	0/0 4/24 11/65 2/12 0/0 0/0	0/0 7/41 8/48 2/12 0/0 0/0	0/0 9/53 8/47 0/0 0/0 0/0

autoDF, autologous dermal fibroblasts.

Quantitative measurements of changes in dermal thickness, which were defined as a distance between skin appendages, showed a significant increase when compared with the baseline, with 44.8  $\pm 2.3\%$  (p <0.05) after 1 month and 62.5  $\pm 6.7\%$  (p <0.05) after 12 months, respectively.

# 4. Discussion

The term 'cell aging' defines the time-related changes of skin fibroblasts with a reduced cell number, decreased

Table 2. Results of visual assessment of clinical effect intensity depending on time, made by three independent study doctors and by patients themselves

Effect	Months after injection of AutoDF							
average score	1, <i>n</i> /%	3, <i>n</i> /%	6, <i>n</i> /%	12, <i>n</i> /%				
Assessment made by three independent doctors								
Excellent	0/0	2/12	4/24	5/29				
Good	2/12	9/53	12/71	12/71				
Satisfactory	15/88	6/35	1/6	0/0				
No effect	0/0	0/0	0/0	0/0				
Negative effect	0/0	0/0	0/0	0/0				
Self-assessment by patients								
Excellent	8/47	10/59	11/65	12/71				
Good	6/35	7/41	6/35	5/29				
Satisfactory	3/18	0/0	0/0	0/0				
No effect	0/0	0/0	0/0	0/0				
Negative effect	0/0	0/0	0/0	0/0				

autoDF, autologous dermal fibroblasts.



Figure 5. Groups of transplanted fibroblasts without signs of mitosis



Figure 6. Skin after SPRS therapy: (A) 1 month, (B) 3 months, (C) 6 months, (D) 12 months. Groups of administered cultured autofibroblasts are marked with arrows. Staining: left column, haematoxylin and eosin; right column, silver nitrate impregnation. Original magnification ×200

motility, proliferation and biosynthetic activity as well as response on growth factors/cytokines (Brown 2004).

As the function of dermal fibroblasts consists in production, organization and renewal of intercellular dermal matrix, the above-mentioned changes in fibroblasts were shown as signs of skin aging. *In vitro* studies have shown that proliferative potential of dermal fibroblasts in an adult person remain on a rather high level during their lifetime: primary cultures obtained from elderly persons (aged 95 years), contained up to 14% of mitotically active fibroblasts (Bayreuther *et al.*, 1992). This was confirmed by the present study as cultures of dermal fibroblasts derived from patients in the age range 45–65 years were characterized by a rather high efficacy of colony formation (45.0  $\pm$ 9.5%) and high expression of primary proteins, and this did not depend on patient's age. It was therefore possible to obtain a number of functionally active cells for therapy from small amount of adult skin biopsy material, regardless of patient's age.

After dermal transplantation of cultured autoDF, their biosynthetic activity remained. Thus, the histological studies revealed prolonged synthetic activity of transplanted autoDF at least for 12 months, which was shown as synthesis of components of the intercellular matrix including young (argyrophil) collagen fibres (Figure 6). Immunohistochemical studies performed using antibodies against CD45 (marker of haematopoietic cells) and vimentin (marker of mesenchymal cells) confirmed that groups of cells observed in the dermis after transplantation of autoDF represent nothing other than fibroblasts.

In the present study, transplanted autoDF were observed in derma without signs of mitotic activity, which confirms the absence of risk for hyperplastic processes when such cells are used.

Changes were observed in dermal microstructure were recorded throughout the observation period and resulted in a progressive increase of dermal thickness that increased by  $62.5 \pm 6.7\%$  in 12 months (p = 0.028).

Clinical monitoring revealed that all 17 patients were satisfied with the clinical results. The improvement in facial skin, which consisted of increased skin firmness, decreased relief, and improved facial colour and contours, was observed by patients 10–14 days after the second procedure of intradermal autoDF administration. A progressive effect was observed and 1 month after autoDF administration, 88% of patients assessed the effect as 'good' and 'excellent'. After 3, 6 and 12 months all patients were satisfied, grading the effect as 'good' or 'excellent'.

The more objective evaluation of wrinkle number and deepness, which was made by independent study doctors on 6-score Facial Fold Assessment Scale, showed the analogous progressive effect and provided values of 86% and 100%, respectively. No adverse events or serious adverse reactions were reported (except for small bruises and swelling that resolved over several days).

The progressive clinical effect was confirmed by instrumental study methods. Thus, the study of facial skin elasticity revealed that intradermal autoDF administration resulted in a progressive increase in values in all measured areas. The maximal increase in facial skin elasticity was observed in the periorbital area, and exceeded the baseline value of 24.0  $\pm$ 4.3% in 6 months, after which it remained almost at the same level up to the end of observation period. In buccal and perioral areas, the progressive, but less pronounced increase of values was also observed in comparison with periorbital area. The statistically insignificant increase in skin elasticity in the perioral area may be associated with rather high baseline values of elasticity in this area (0.7 arbitrary units, while in periorbital area it was only 0.44 arbitrary units; Figure 2).

The general tendency towards progressive effect after autoDF administration was also shown with measurements from the VISIA photometric system and optic profilometry. The less pronounced decrease in wrinkle deepness, which was observed in perioral area, may be associated with anatomical particularities of the zone, in particular, the presence of an active, orbicular muscle of mouth, closely attached to the skin, which was able to greatly influence facial skin microrelief.

# 5. Conclusion

The clinical observations showed the safety and clinical efficacy of autoDF for the correction of changes in aging skin. After transplantation to skin, the biosynthetic potency of cultured autoDF remained, and they actively produced the components of dermal intercellular matrix at least for 12 months. As a result, remodelling of dermal microstructure was observed, which was demonstrated by an increased collagen fibre content of and increased thickness. Clinically significant changes of dermal microstructure were shown as increased skin turgor, reduced number and deepness of wrinkles, and improved face colour and contours.

# **Conflict of interest**

The authors have declared that there is no conflict of interest.

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# Supporting information on the internet

The following supporting information may be found in the online version of this article:

Figure S1. Scheme of AutoDF intradermal injection.

Figure S2. Immunohistochemical staining with antibodies against vimentin.

Figure S3. Immunohistochemical staining with antibodies against CD45.