

DEFORMATION PROPERTIES OF MASTOID AREA SKIN**STEBLOVSKIY D.V.*, AVETIKOV D.S., LOKES K.P., POPOVICH I.Y., BOYCHENKO O.N.**

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*Received 12/02/2018; accepted for printing 18/07/2018***ABSTRACT**

The mechanical properties of the skin have been studied in vivo and in vitro using various test methods. It has been found that with age, the skin undergoes considerably less deformation at the same voltage, which is probably due to the change of collagen and elastin nets.

Two-dimensional biomechanical behavior and the content of collagen in human skin samples from different areas of topical localization were studied. The minimum and maximum reduction axes were determined after removal and their connection with Langer's lines. The experimental equipment was designed to keep the geometry and measure loads acting perpendicular to the circle of skin samples. Using in vivo geometry of samples as a base, various deformations were applied. After the voltage relaxation, the final values of the voltage were taken and compared with the deformations. It was found that the axes of maximum and minimum stress do not coincide with the Langer's lines, and rotated 10°.

This article describes the structure of the mastoid area skin. After the objective morphological studies, we came to the conclusion that during the surgery on the mastoid region, namely cosmetic otoplasty and lower rhytidectomy, the length of stretching skin-fat grafts must be taken into account. No visible lesions were found in their deformation in the range of 5-10 mm. This is the optimal minimum and maximum limit of biomechanical parameters in such interventions. Pathological changes primarily in the form of balloon degeneration were observed at the 15-20 mm flap tension, indicating a greater likelihood of necrosis in the postoperative period. Deformation of the skin-fat grafts of 25 mm leads to irreversible changes and hemodynamic disorders, thus flap stretching within these parameters is unacceptable.

The aim of this study was to investigate the morphological features of skin-fatty grafts of mastoid area at various stages of deformation. In the experiment we used 30 skin-fatty grafts taken from the patients with roughness during cosmetic otoplasty. These pieces were elongated at 5, 10, 15, 20, 25 mm respectively; they were colored by hematoxylin-eosin as well as van Gieson stain.

KEYWORDS: skin, deformation, mastoid area, stretching.**INTRODUCTION**

At the moment, there are a lot of researches devoted to various skin-related problems, among them a number of works on defining the age changes in the skin. Some studies at the microscopic level show the tissue aging due to slowing metabolism and oppression synthesis of collagen and elastin fibers. But there are no literature data on studies of the changes in skin thickness and

density of collagen fibers and their dependence on sex and age [*Santoni-Rugiu P, Sykes J, 2007*].

With the help of light and raster electron microscopy an attempt to study the structure of the dermis in different areas of the head and to show the dependence of the biomechanical properties of the skin in these regions on the features the fibrous structures was made [*Taber L, 2004*].

The mechanical properties of the skin have been studied *in vivo* and *in vitro* using various test methods. It has been found that with age, the skin undergoes considerably less deformation at the same voltage, which is probably due to the change of collagen and elastin nets [*Kanitakis J, 2002*].

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Many authors investigated *in vivo* various visco-elastic parameters that describe the mechanical properties of human skin at different ages. The results of the study showed that with the age of a person the skin retains its thickness and elongation, in contrast to its elasticity or the ability to recover, which decreases since the early age. The viscous part of the deformation is constant throughout life, while the relaxation time for creep is linearly decreasing over the years [Avetikov D, Steblovsuy D, 2013].

The scientists studied two-dimensional biomechanical behavior and the content of collagen in human skin samples from different areas of topical localization. The minimum and maximum reduction axes were determined after removal and their connection with Langer's lines. The experimental equipment was designed to keep the geometry and measure loads acting perpendicular to the circle of skin samples. Using *in vivo* geometry of samples as a base, various deformations were applied. After the voltage relaxation, the final values of the voltage were taken and compared with the deformations. It was found that the axes of maximum and minimum stress do not coincide with the Langer's lines, and rotated 10° [Moran C et al., 1995].

Some authors carried out research on blood circulation when changing external pressure on the skin. In work on the surface of the human skin was pressurized and using laser dopplerometry, data was obtained on the discontinuation of blood circulation in the skin: it is 8 kPa and 9.3 kPa, respectively [Liu J et al., 2010].

Analyzing literary data, we can conclude that the accumulated extensive experience in the study of skin characteristics, conducted a large number of experiments, including *in vivo*, widely used acoustic (non-invasive) methods for determining the characteristics of soft tissues [Gundiah N et al., 2007].

However, there is a number of unresolved issues that have not been properly developed. The problem is to choose a mathematical model that adequately describes the behavior of the skin during deformation [Wolman M, Kasten F, 2006].

At present the experience of modern face and ear aesthetic surgery requires techniques to optimize recovery and mobilize skin-fat grafts within a particular area and depth of their detachment [Bao G, Suresh S, 2003].

This especially concerns the mucous membrane, in which the main stages of the lower rhytidectomy and cosmetic otoplasty are carried out. The interest of researchers is conditioned by a larger number of patients who showed rapid removal of involuntional ptosis, lop ear as well as the evolution of modern surgical technologies [Gambarotta L et al., 2005].

In order to achieve optimal performance of cosmetic otoplasty and lower rhytidectomy; to minimize postoperative complications and lack of manual skills of a surgeon, it is necessary to know perfectly the morphofunctional features of the layered structure of topographic anatomical sites in which surgical intervention is performed, with the determination of the biomechanical and morphological properties of the skin [Reihnsner R, Balogh J, 1995].

According to recent statistics published in contemporary journals, pathological scars occur in 10% of the total world population. Therefore, the formation of optimal aesthetic scar was and remains a major problem in plastic and maxillofacial surgery [Avetikov D et al., 2015].

The aim of this study to investigate the morphological features of skin-fatty grafts of mastoid area at various stages of deformation.

MATERIAL AND METHODS

In the experiment we used 30 skin-fatty grafts taken from the patients with roughness during cosmetic otoplasty. These pieces were elongated at 5, 10, 15, 20, 25 mm respectively; they were colored by hematoxylin-eosin as well as van Gieson stain. Experiment to determine deformation of skin properties fat scraps is carried out on the base of Department of Medical Informatics, Medical and Biological Physics. Biomechanical studies were conducted on the deformation installation MKR-1. One end of the flap was fixed in moving, the other - in a stationary position.

The obtained data were processed by the method of variation statistics. All of these parameters were determined using the Excel spreadsheet and the Microsoft Excel program package. As to nonparametric methods in determining the probabilities of the research indices, we used the U-criterion of Wilcoxon-Mann-Whitney. Differences were considered probable at $p \leq 0.05$.

RESULTS

Processing of the results was carried out by conventional statistical methods. During the investigation of microscopic skin preparations of mastoid area after stretching within 5 mm, there are minimum, barely noticeable differences from the intact skin of respective anatomical area. So, basal, ribbed, granular and horny epidermis layers are clearly differentiated, its structural organization was virtually no different from previously described. However, a slight increase of intraepithelial lymphocytes in the basal and lower parts of the horny layer, which occurred mainly in the peripheral parts of the skin graft should be noted. In our opinion, it was a response to mechanical force. A little more than in normal epitheliocytes of horny layer, it was detected mainly perinuclear "optically empty" vacuoles, which indicated the presence of hydropic dystrophy. However, the presence of these formations in such small amounts should not be regarded as a pathological process (Fig. 1).

During the studied intact skin stretching to the above value, the superficial papillary and the horny layers located underneath were clearly differentiated in the dermis. The connective tissue papillae in the form of a truncated and pointed cone have prevailed in the surface layer. The papillae that had the same size at the base and the tip, with close to rectangular shape, were rare. The lymphocytes and plasma cells with mature fibroblasts were defined among the cellular elements of papillary dermis. Their number was slightly higher than the same in-



FIGURE 1. The structure of skin graft after stretching within 5 mm. Microslide. The color by hemotohylin-eosin. O. 20x, oc. 7x. 1 – epitheliocytes with symptoms of hydropic dystrophy; 2 – intraepithelial lymphocytes; 3 – connective tissue papillae; 4 – lymphoid cellular elements in the papillary layer of the dermis

indicator in intact skin, while in some cases lymphoid cells tended to form the groups of fine focal clusters that were more clearly expressed in the peripheral parts of the skin graft (Fig. 1).

During the study of microscopic skin preparations of mastoid area that were stretched at 10 mm, changes become more apparent compared to intact skin. The manifestation of pathological processes was less noticeable in the epidermis than in the dermis. The increase of cells with signs of hydropic degeneration in the thorny layer should be noted in the experimental group. In some cases, epitheliocytes had pyknotic corrugation of cores. The described morphological pattern should be regarded as a pathological process involving a violation of the trophic epithelial cells, which can be due to mechanical action. In some cases, dystrophic changes of the epithelial cells were visualized not only in the horny but in the basal layer also (Fig. 2).

During the detailed investigation of the surface layer of the dermis, some changes at connective tissue papillae were noted. Thus, compared to intact skin and preliminary experimental group, the slight increase of relative number of papillae with the shape of a truncated cone and papillae, with the shapes close to rectangular was observed. Occasionally, smooth epidermal-dermal papillae verge of flattening was marked in some areas. We have found the explanation of this circumstance by

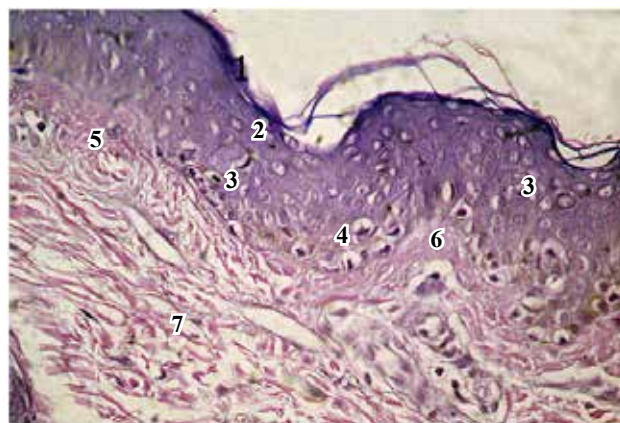


FIGURE 2. The structure of the skin flap after stretching within 10 mm. Microslide. The color by hematoxylin-eosin. O. 30x, oc. 7x. 1 – the horny layer of the epidermis; 2 – granular layer of the epidermis; 3 – epitheliocytes of horny layer with symptoms of hydropic dystrophy; 4 – degenerative changes in cells of the basal layer of the epidermis; 5 – plot of smoothing of the epidermal-dermal border; 6 – connective tissue papilla in the form of a pointed cone; 7 – reticular layer of the dermis

studying the specimens of papillary dermis stained by picro-fuchsin at high magnification on light microscope. However, some refinement bundles of collagen fibers in comparison with previous experimental group were determined. But the nature of their location relatively to papillae length had longitudinal character in most cases observed. The elastic fibers that were found in connective tissue papilla had no structure difference from those in prior experimental groups. They were focused respectively on the progress of collagen fibers in most cases. The features of its location were described above in the experimental group. Thus, some changes in the nature of the layout and topography of fibrillar component in connective tissue of papilla dermis were determined. It performed shaping function and provided mechanical properties, resulting in some changes in the shape of papillae, and flattening in some areas on the border between the epidermis and dermis.

At the same time, significant changes in the cellular composition of connective papillae were not observed. The lymphocytes and plasma cells located in areas of epidermal - dermal border, with fine focal infiltrates, mainly in the peripheral parts of the skin graft together with cellular fibroblastic elements were observed.

In some observations, metabolic microvasculature of blood vessels with symptoms of mild hyperemia were noted. It had a functional character and was caused as a result of mechanical action on the skin. The small groups of lymphocytes and isolated macrophages were established around described microvessels. Still, significant changes in the structure of the appendages were not found.

The study of the skin-fatty flap structure after 15 mm stretching demonstrated the aggravation of pathological processes in the surface epithelium of the skin and in the dermis. Thus, the progressive increase of cells in the state hydropic dystrophy was observed in the horny layer of the epidermis in the experimental group. The cytoplasmic vacuoles with fluid, occupying almost the entire space of the cytoplasm were visualized in some of them. This morphological pattern showed extreme severity measure of hydropic dystrophy – the development of balloon dystrophy, which is known as morphological equivalent of focal necrosis. The pyknotic shriveled kernels in epitheliocytes were often manifested.

Dystrophic changes of epithelial cells, the presence of intraepithelial lymphocytes in the basal layer of the epidermis were found. It should be noted that a slight decrease of the number of mitotic figures, may indirectly indicate a decrease of regenerator ability of epithelial layer, which is a consequence of pathological processes developed in an appropriate tissue.

Changes of papillary dermis in connective tissue were virtually identical to the results in 10 mm tension of skin-fatty flap. Thus, an increase of relative number of papillae with truncated cone and close to rectangular forms was noted. Occasionally the epidermal-dermal border lost undulate nature in the central parts of the skin. The thinning of collagen fibers and violation of their normal topography were marked in the papillary dermis layer. Similar changes occurred in the elastic fibrils.

Unlike previous experimental group, some changes in metabolic circulatory vessels of micro-circulatory system should be noted. Thus, phenomena of desolation were detected in some arterioles and capillaries which indicated the development of ischemia. Described changes were more marked in peripheral parts of the skin graft. The above changes in blood microvessels were developed due to mechanical action on the skin. It impeded the blood flow as well as slowed blood flow-out from some compartments of the surface layer of the dermis.

The increase of vascular permeability caused a relative increase of hematogenous origin cells in the connective tissue papilla - macrophage at different stages of differentiation, lymphocytes and plasma cells. These cellular elements were located in the area of diffuse epidermal-dermal border and formed fine focal infiltrates, mainly at perivascular areas in the peripheral parts of skin graft.

The changes in the reticular layer of the dermis, unlike the previous group, were found at longitudinally arranged collagen fibers and those that had oblique, tangential direction and characterized as a rather considerable length thinning sites and sites that had an uneven thickness.

Special attention should also be paid to changes in the blood microvessels, which were generally located in the papillary layer of the dermis. The deflated arterial microvessels were noted in the peripheral parts of the skin graft; forming elements

were absent in its lumen. Most venous microvessels, in contrast, were characterized by excessive blood flow and increased vascular permeability. It was an evidence of the perivascular edema, minor bleeding and fine focal cell infiltrates in supporting spaces. The sludge-phenomenon and the formation of small blood clots occurred in some microvessels. At the same time, pathological changes in the sweat and sebaceous glands at these limits of experimental deformation in all parts of the skin graft were not detected (Fig. 3).

The study of microscopic preparations of skin stretching within 20 mm at mastoid area can detect amplification described above and the emergence of qualitatively new lesions in the epidermis and the dermis. In particular, the mitotic activity of cambial cell elements of the epidermis decreased, the cause of which was discussed earlier. The expressed hydroptic, in some cases balloon dystrophy in the horny layer of the epidermis, some changes were observed in the granular layer.

Thus, in contrast to the intact skin of the above range of deformation, the areas with cellular elements of the granular layer arranged in two rows were visualized at epidermis. At the same time, the quite large areas of complete absence of these cellular elements, there the stratum corneum located directly over thorny cells were present. Several observations have determined fairly large area of keratolysis - detachment of the horny layer of the epidermis layer with proper formation of very

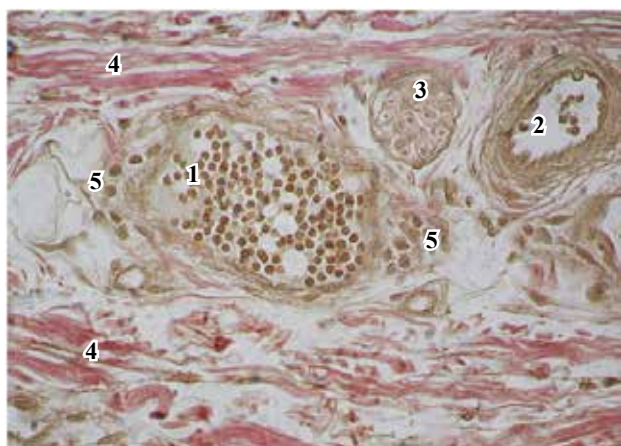


FIGURE 3. The structure of skin graft after stretching within 15 mm. Microslide. The color by van Gizon. O. 30x, oc. 7x. 1 – veins with events of plethora; 2 – arterial microvessel; 3 – nerve trunk; 4 – bundles of collagen fibers at reticular dermis; 5 – zone of perivascular edema with cell infiltrates

large subcutaneous cavities. Periodically, the focal thickening of the epithelial layer formed by increasing the number of rows granuloosa cell was occurred at peripheral areas of skin graft. The zones of thickening of the horny layer were proved in these parts of the skin graft. The so-called parakeratotic, pyknotic cells containing the modified rod-shaped core were marked, it was the evidence of focal parakeratosis. Significant changes were also occurred in the surface, papillary layer of the dermis. Along with papillae with the shape of a truncated cone, flattened nipples, nipples rectangular and connective tissue papillae with modified wrong, polymorphic form substantially different from each other metric were often manifested.

The microcirculation disorders in papillary layer of the dermis were also noted almost everywhere that was unlike the previous experimental group. First, hemodynamic disorders were characterized by arterial spasm of microvessels and desolation. The uneven blood flow and increased vascular permeability were found in exchange and capacitive microvessels. The perivascular fine focal hemorrhages were quite often proved, mostly marked in peripheral parts of the skin graft.

The disorders of microcirculation occurred in all parts of the skin graft and were characterized by the changes that were similar to those in the papillary layer, unlike the previous experimental group. An increase of the number of mast cells at paravascular spaces should be also noted. Sometimes, degranulation was present accompanied by the release of biologically active interstitial substances. The changes in the sweat glands occurred in the experimental group. Thus, ductless glands became somewhat convoluted in some cases, which in its turn led to the narrowing of its lumen. It was the secretion accumulation in secretory parts of the glands which led to the expansion of their lumen. Such change in the terminal portion of the sweat glands was rather associated with difficulty in outflow secret by the deformed excretory duct. The sebaceous glands have retained typical structure in all observations.

The study of the structure of skin graft after stretching within 25 mm demonstrated severe pathological changes in the epidermis and dermis that were irreversible in most cases. The changes that were observed in the surface epithelium, largely re-

sembled the results of tensile of skin graft within 20 mm. However, a number of observations were more marked. Thus, the keratolysis areas were observed more often. The parakeratotic cells were almost always determined in the corneum layer. The hydropic and in some cases focal balloon dystrophy were present in ribbed layer. The intercellular spaces were expanded due to excessive accumulation of extracellular fluid affecting the intercellular communications between individual epithelial cells.

However, the most significant changes were observed in the dermis. First of all, the polymorphism of papillae, which was more marked than in preparations with less deformation outside should be noted. Very significant length areas were also always proved. The boundary between the epidermis and dermis looked like almost straight lines in these areas due to marked flattening of papillae (Fig. 4).

In a detailed study of the internal structure of the connective tissue papillae on preparations stained by picrofuchsin not only thinning and abuse typical spatial orientation bundles of collagen fibers were proved. Also, severances of the last that had taken place in the apical and basal portions of papillae. It should be noted that the largest number of severed fibers were determined at deformed and flattened papilla and in papilla that retain the typical structure of collagen fibrils. Besides, the change of tinctorial properties of collagen fibers was noted, as a result painted in orange, sometimes yellow. That has indicated they development of irreversible dystrophic process – fibrinoid swelling.

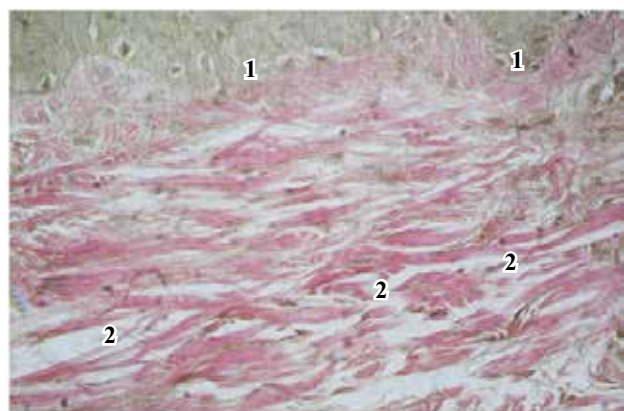


FIGURE 4. The structure of the skin graft after stretching within 25 mm. Microslide. The color by van Gizon. O. 30x, oc. 7x. 1 – the border between the epidermis and dermis; 2 – longitudinal bundles of collagen fibers with destructive changes at reticular dermis

The cell infiltrates, consisting mainly of macrophages and lymphocytes were marked around the areas of dystrophic and fragmented collagen fibers. The presence of these formations may indicate the formation of immune reaction to necrotic fibrous structure.

Different kinds of hemodynamic disorders were found in connective tissue papillae, as in previous preparations of blood microvessels that had manifested mainly microvascular arterial spasm and symptoms of anemia. Fine focal perivascular hemorrhages located in the peripheral and in the central parts of the skin graft occurred periodically.

Periodically, the destruction of collagen fibers was observed in the reticular layer of the dermis at staining by picro-fuchsin. This process was more often seen in longitudinally oriented fibrils while in tangentially oriented fibers destructive processes were significantly less marked. At the same time, a significant increase in the relative number of longitudinal bundles of collagen fibers and therefore reduce of the number of tangential should be noted (Fig. 5). Described restriction of fibrillar component of the dermis, in our opinion, is associated with the mechanical stress caused by tension. In some cases, violation of integrity was determined at thinned dramatically elastic fibers, parallel to the longitudinal collagen fibers.

As in the papillary layer, microcirculation disorders were manifested by specific microvascular spasm, their desolation and perivascular hemorrhages were present in reticular layer of the dermis. It should be noted that unlike the previous

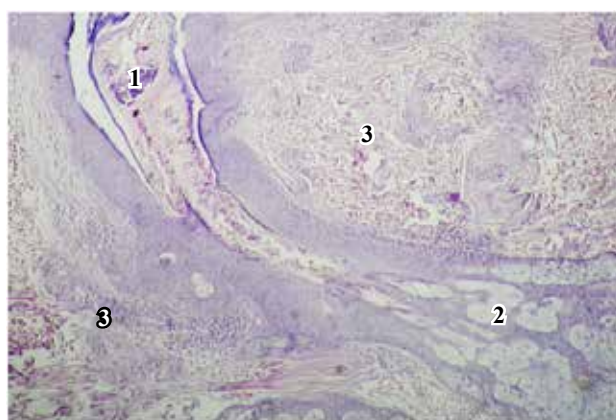


FIGURE 5. The structure of the sebaceous glands in the skin graft after stretching to 25 mm. Microslide. The color by hematoxylin-eosin. O, 40x, oc. 7x 1 – secret in the extended excretory duct; 2 – unmodified secretory departments; 3 – connective tissue of reticular dermis

experimental group, where stretch skin graft was performed to 20 mm, neglect of capillaries and arterioles spasm was detected in both surface and deep layers of the dermis.

CONCLUSION

Thus, after the objective morphological studies we came to the conclusion that during the surgery in the mastoid region, namely cosmetic otoplasty and lower rhytidectomy the length of stretching skin-fat grafts must be taken into account. No vis-

ible lesions of deformation in the range of 5-10 mm were found. This is the optimal minimum and maximum limit of biomechanical parameters during such interventions. *Pathological changes primarily in the form of balloon degeneration were observed at the 15-20 mm flap tension, indicating a greater likelihood of necrosis in the postoperative period.* The deformation of the skin-fat grafts of 25 mm leads to irreversible changes and hemodynamic disorders, thus flap stretching within these parameters is unacceptable.

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