Scientific Research Publishing

Open Journal of Ophthalmology, 2023, 13, 122-135
https://www.scirp.org/journal/ojoph
ISSN Online: 2165-7416
ISSN Print: 2165-7408

Ultrastructural and Immunohistochemical Characteristics of Corneal Lenticule Extracted during Correction of Residual Myopia in the Long-Term Period after SMILE

Olesya Pisarevskaya[1*], Boris Malyugin[2], Andrey Shchuko[1,3,4], Tatiana Iureva[1,3,4], Lyudmila Vasilieva[3], Nikolay Sudakov[5], Igor Klimenkov[5], Elena Likhoshvay[5]

[1]Irkutsk Branch of S. Fyodorov "Eye Microsurgery" Federal State Institution of Ministry of Health of Russian Federation, Irkutsk, Russia
[2]S. Fyodorov "Eye Microsurgery" Federal State Institution of Ministry of Health of Russian Federation, Moscow, Russia
[3]Irkutsk State Medical University of Ministry of Health of Russian Federation, Irkutsk, Russia
[4]Irkutsk Branch of Russian State Medical Academy of Postgraduate Education of Ministry of Health of Russian Federation, Irkutsk, Russia
[5]Limnological Institute, Siberian Branch of Russian Academy of Sciences, Irkutsk, Russia
Email: *anna.val.kripak@gmail.com

How to cite this paper: Pisarevskaya, O., Malyugin, B., Shchuko, A., Iureva, T., Vasilieva, L., Sudakov, N., Klimenkov, I. and Likhoshvay, E. (2023) Ultrastructural and Immunohistochemical Characteristics of Corneal Lenticule Extracted during Correction of Residual Myopia in the Long-Term Period after SMILE. *Open Journal of Ophthalmology*, **13**, 122-135.
https://doi.org/10.4236/ojoph.2023.131012

Received: October 26, 2022
Accepted: February 20, 2023
Published: February 23, 2023

Abstract

Purpose: To evaluate ultrastructural characteristics of lenticule surface extracted during correction of residual myopia in patients after small-incision lenticule extraction (SMILE). **Methods and material:** This study had a prospective, consecutive, comparative design. Sixteen patients (16 eyes) underwent additional intervention for residual myopia correction after SMILE. 16 specimens of removed lenticules underwent morphological examination. Markers and reagents were used to determine actin microfilaments, neutral fats and cell nuclei. The tissue was analyzed in layers in 2D slices form, volumetric Z-stacks, or selected areas were formed in orthogonal projections. The surface of the extracted lenticule was analyzed using scanning electron microscopy. Patients' refractive outcomes were measured postoperatively (1 day; 1 and 3 months). **Results:** Postoperatively uncorrected distance visual acuity (20/20 or better) was in 100% cases 3 months after surgery. Ultrastructural studies have shown the difference in surfaces of the newly formed lenticule. Structural changes of the posterior lenticule surface were characterized by ruptures of collagen fibers on its surface, degenerative changes in keratocytes with signs of colliquation necrosis, cell apoptosis and F-actin in cell cytoplasm. **Conclusion:** Collagen fibers are immersed in the stroma on the anterior surface of the lenticule. There is no complete structure restoration of col-

lagen fibers explaining the lack of tight adhesion of anterior and posterior surfaces of the intrastromal space even in the long-term postoperative period. There are no degenerative changes of keratocytes on the anterior lenticule surface, that is, their changes in SMILE are reversible in most cases.

Keywords

SMILE, Residual Myopia Correction, Lenticule, Ultrastromal and Immunohistochemical Analysis

1. Introduction

Laser technology is one of leaders in ophthalmology development. Surgical correction of myopia is on the top among all refractive surgeries. Dr. Kurtz at the beginning of 90 ties invented femtosecond laser technology. It has revolutionized modern ophthalmic surgery providing high precision minimizing risks of inflammation [1].

In 2006, a group of German ophthalmologists led by Walter Sekundo and Marcus Blum, introduced a new approach for myopia and astigmatism correction called femtosecond lenticule extraction or refractive lenticule extraction (FLEx/ReLEx). Two corneal cuts (posterior and anterior) are involved in the FLEx procedure, creating a lenticule that is ultimately removed. In 2009 to correct refractive errors, the existing technology was improved and got a new name—SMILE (Small-Incision Lenticule Extraction). The lenticular interfaces are separated by a dissector passing through a small incision (2.0 - 4.0 mm) allowing the lenticule to be extracted [2].

To date, clinical studies have proven the main advantages of this technology: the stability of the refractive effect and corneal biomechanics, ensured by the native location preservation of collagen fibers, the structure and functional activity of keratocytes involved in the synthesis and maintenance of the extracellular matrix [3] [4] [5] [6] [7].

Studies of wound healing after SMILE conducted on a rabbit model showed that femtosecond laser surgeries are accompanied by moderate expression of Ki-67, a cell marker of fibronectin, as well as antibodies to CD11b, which, on the one hand, are involved in the creation of a temporary matrix for the migration of epithelial cells or keratocytes to the injury zone, and on the other, reflect the degree of inflammatory reaction and proliferation [8] [9].

Studies of corneal lenticules extracted in humans revealed a decrease in the concentration of proinflammatory cytokines (IL-1 b, TNFα), and TUNEL-positive keratocytes reflecting their death by apoptosis [10]. Experimental studies have established the role of cellular mechanisms in the generation of fibrous tissue at the site of femtosecond laser exposure [11].

However, the mechanisms of postoperative corneal wound healing, which is an avascular tissue and has a homogeneous cellular composition, have not been

well studied. The results of researches obtained to date do not allow us answering questions about the causes of the formation of intrastromal opacities and regression of the refractive effect after SMILE [12] [13] [14].

The introduction of a new approach for correcting residual myopia based on the creation and removal of a new lenticule through a previously formed access allowed studying the regularities of corneal wound healing both in early and long-term postoperative periods [15].

The purpose of this study was to conduct ultrastructural and immunohistochemical studies of the lenticule extracted during laser correction of residual myopia in patients operated earlier by the SMILE method.

2. Methods and Material

This study had a prospective, consecutive, comparative design. Sixteen patients (16 eyes) underwent additional intervention for residual myopia correction after SMILE from May 2019 to December 2019. The study was reviewed and approved by the Local Ethic Committee of Irkutsk Branch of S. Fyodorov Eye Microsurgery Federal State Institution of Ministry of Health of the Russian Federation (Protocol No. 23/2, 23/04/2018).

Inclusion criteria: low residual myopia after SMILE, not earlier than 12 and not later than 18 months after the first Smile surgery; minimal stroma thickness after correction of residual myopia at least 280.

Exclusion criteria: corneal opacity, epithelial in growth in the access zone, an increase in the axial length of the eyeball, corneal thickness less than 390 - 400 microns, eye inflammatory diseases, mental illnesses and allergic reactions.

All patients had preoperative evaluation of the objective and manifest refraction, visual acuity (Snellen charts), slit lamp examination. Corneal thickness and topography were assessed by Pentacam (Oculus Inc., Germany). OCT AVANTI RTVue XR with anterior segment (Optovue Inc, Ca., USA) was used to assess the cornea condition.

One surgeon (O.P.) performed all operations by a femtosecond laser VisuMax 500 kHz (Carl Zeiss Meditec AG), software version 2.0, with parameters: lenticule - spot distance and tracking spacing (4.5/4.5μm), lenticule side-cut - 2.5/2.5 μm. To prevent complications during the surgery the corneal neutral optical layer was increased from 15 μm to 30 μm. Cap thickness settings were reduced by 5 μm from the baseline, the optical zone was reduced by 0.2 mm from the baseline. The corneal access settings were the same, since they were not used in this surgery.

The surgery was carried out using an expert mode that allows controlling all stages of the surgery. At the stage of formation of the lenticule posterior surface and the side cut, energy remained at the same established level - 36 (180 nJ). The energy changed to a value of 35 during the transition of the operation to the stage of formation of the corneal flap and corneal incision. When the energy transferred to another level, the laser stopped.

At first the lenticule posterior surface and the side cut were formed. Then la-

ser stopped. After that, a thin spatula was inserted via the corneal incision to get into the intrastromal space that was easily differentiated. By the same spatula we made the blunt dissection of lenticule posterior surface. The lenticule was grasped with forceps (23G/0.6mm, DORC, Netherlands) and extracted from the cornea through a previously formed supertemporal corneal incision. After that, we flushed the intrastromal pocket with a balanced salt solution.

Postoperatively, moxifloxacin eye drops (Alcon, Alcon Laboratories Inc, USA) 0.5% were used 4 times (7 days); dexamethasone eye drops (Oftan-dexamethasone (Santen Pharmaceutical Co, Ltd., Finland), 4 times a day (14 days); sodium hyaluronate eye drops (Ursapharm Arzneimittel GmbH, Saarbrucken, Germany), 4 times a day (30 days).

Postoperative examination included objective and manifest refraction, visual acuity, and slit-lamp examination (1 day, 1 and 3 months after surgery). Postoperative follow up was 3 months.

3. Microstudy of the Extracted Lenticules

To prevent tissue curling, we placed the lenticule on a filter paper with the front side up. The lenticule specimens were fixed in 2.5% glutaraldehyde (Sigma-aldrich, USA) on a 0.1 M phosphate buffer (pH 7.4) for 1 hour, washed with the same buffer (3 times for 10 minutes), fixed for 12 hours in 2% osmium tetrachloride at +4˚C (Sigma-aldrich, USA) and poured into epoxy resin. During the pouring process, the samples were successively impregnated in intermediate resin and acetone solutions (with an increasing proportion of resin) in the following ratios 1:3, 1:2, 1:1 (3 hours in each solution). Further, after extraction from the last mixture, the tissue pieces were freed from the previous resin, placed in capsules with a fresh resin mixture with a DMP-30 catalyst and placed in a thermostat at a temperature of +60˚C. After polymerization, ultrathin sections (thickness 70 - 80 nm) were made on the Ultracut R ultramicrotome (Leica, Switzerland), which, after contrasting in lead citrate according to Reynolds (Reynolds, 1963), were examined using a transmission electron microscope (Leo 906 E, Zeiss, Germany). With the help of scanning electron microscope (SEM) (Quanta 200 (FEI Company, USA) 2 lenticule sides were distinguished); back side is the surface worked out by a femtosecond laser during the formation of a new lenticule during the correction of residual myopia in the long-term period after first SMILE, the anterior surface was formed during the first SMILE surgery.

To study the surface structures, lenticules were fixed according to the above procedure and dehydrated in a graded series of aqueous ethanol solution (30%, 50%, 70%, 96% and 100%). After dehydration, the material was mounted on aluminum tables; gold was sprayed on the SCD 004 (Balzers) apparatus and imaged with SEM.

For further laser confocal microscopy, during 15 min lenticules were washed in medium 199 and then fixed in 4% paraformaldehyde (Sigma-Aldrich, USA).

For visualization of actin microfilaments, after fixation the samples were fixed in 1% Triton X100 (Sigma-Aldrich, USA) for 20 min. and treated with phalloi-

din-FITC (ex/em 490/525nm) for 40 minutes.

To detect neutral lipids, after fixation the samples were treated with a lipophilic reagent staining neutral lipids, Nile Red (Sigma-Aldrich, USA, ex/em 490/525nm) during 15 min.

To detect nuclei of the cell, the samples were stained for 15 min with DAPI (Sigma-Aldrich, USA, ex/em 340/488nm), 0.5 mcg/ml.

After washing, we enclosed the samples in ProLong® Gold antifade reagent (Thermo Fisher Scientific Inc., USA) and evaluated by LSM 710 (Zeiss, Germany); we used lasers (channel 1 - 405 nm, Ch1: 410 - 507; channel 2 - 488 nm, Ch2: 500 - 749).

Two programs were used (ZEN 2010 (Zeiss, Germany) and Imaris® Bitplane 7.2.3) to study the images obtained. The tissue was analyzed in layers in the form of 2D slices, volumetric Z-stacks, or by forming orthogonal projections of chosen areas. For objective quantitative assessment of the volumes occupied by the products of cytochemical reactions (cell nuclei and lipid content), individual fragments of lenticules were used.

Z-stacks of lenticule images were divided into separate, smaller (1×106 mm^3) fragments where the volumes occupied by the fluorescent marker were determined.

All data collected were counted in Excel 2016 (Microsoft Inc., Redmond, WA). To compare differences Mann-Whitney U test was used (Statistica 10).

4. Results

16 patients (16 eyes) were included (10 females/6 males). Mean age 26 ± 5.6 years (min. 22, max 35). Preoperative UDVA was −0.1 ± 0.02. Sphere and cylindrical errors (−2.36 ± 0.53 D and −0.87 ± 0.18 D). Mean SE was −2.79 D. Preoperative CDVA was 1.0 (range, 0.9 to 1.1).

UDVA of 20/20 or better was achieved at 1 day (82.6%), 1 month (96.4%) and 3 months (100%). At 1 day postoperatively, UDVA was 0.85 ± 0.06 (0.8 to 1.0), at 1 and 3 months after surgery was 0.95 ± 0.03 and 0.97 ± 0.02 respectively. Refractive parameters at 1 day postoperatively: sphere and cylindrical errors (+0.62 ± 0.88 D and −0.37 ± 0.17 D), mean SE 0.80 D; at 1 month, sphere and cylindrical errors (+0.5 ± 0.35 D and −0.25 ± 0.01 D; mean SE - 0.63 D; at 3 months, all eyes had ±0.25 D. Refraction became stable within 1 month after surgery. Safety of residual myopia correction in the long-term period after first SMILE surgery: 10 eyes (63%) had unchanged CDVA; 5 eyes (31%) gained 1 line and 1 eye (7%) lost 1 line. 3 months postoperatively, 13 eyes (81%) had an unchanged CDVA; 2 eyes (12.5%) gained 1 line and 1 eye (6.5%) gained more than 1 line.

4.1. Ultrastructural Lenticules Analysis

92 images were obtained with a scanning electron microscope (Quanta 200, FEI Company); 82 images were obtained with a laser scanning microscope LSM 710 (Zeiss Germany); 14 video files, 56 source files (Z-stacks); 122 images were obtained with a transmission microscope 181.

4.2. Anterior Surface Assessment

Scanning electron microscopy revealed the absence of needle structures on the anterior lenticule surface—the lower boundary of the intrastromal space formed during SMILE surgery. A regular folding was revealed, apparently corresponding to the position of the collagen bundles interwoven into the corneal stroma (**Figure 1(a)**, **Figure 1(b)**). In the thickness of the collagen bundles, the detected keratocytes had morphological signs typical to native cells; cytoskeletal components were clearly detected in their cytoplasm, keratocytes with signs of dystrophy were not detected (**Figure 1(c)**).

Laser confocal microscopy and specific staining of the lenticule with Nile Red binding to neutral lipids revealed the presence of small lipid droplets in keratocytes. These inclusions had a predominantly oblong shape, the conjugate diameters of these ellipsoids were 5.58 (3.8475; 6.1975) μm and 0.86 (0.65; 1.2975) μm, respectively. There were also spherical lipid droplets with a diameter of 1.18 (0.815; 1.645) μm. The identified inclusions were located in keratocytes perinuclearly, or in one of the processes of the cell (**Figure 2(a)**, **Figure 2(b)**). On some preparations, lipid droplets were found in the thickness of the extracellular matrix. Lipid components contained in the lenticule were found for the first time and need further studying.

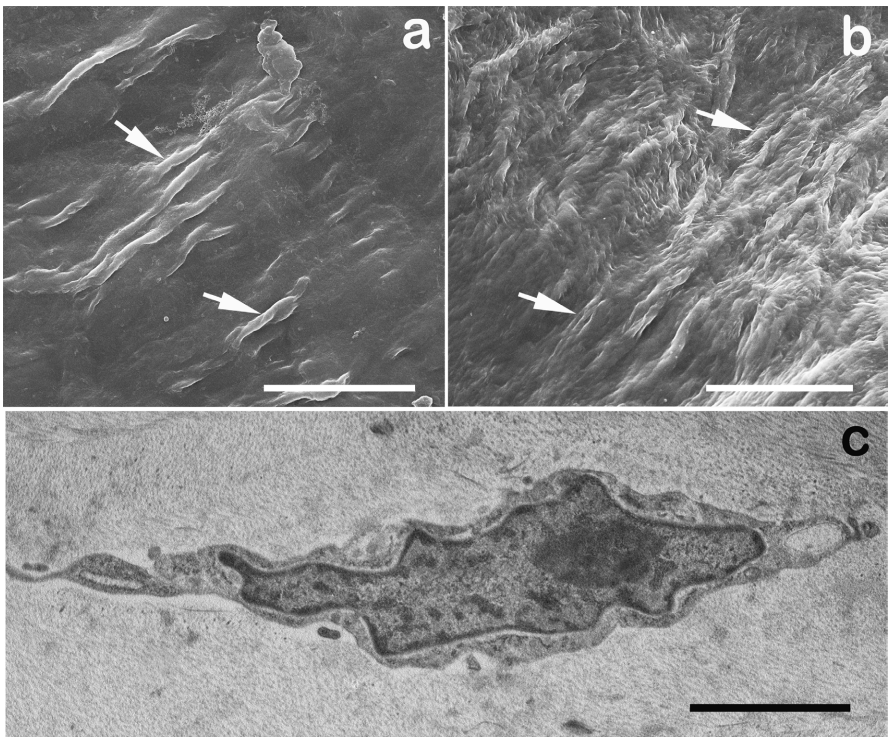

Figure 1. The condition of the anterior surface of lenticule extracted during the correction of residual myopia in the long-term period after SMILE surgery; ((a), (b)) scanning microscopy; (c) transmission microscopy. ((a), (b)) ultrastructure of lenticule surface, formed with extracellular matrix of keratocytes; collagen fiber folds pointed with arrows; (c) single keratocyte in the depth of collagen fibers. Scale bars: (a) 20 mkm; (b) 100 mkm; (c) 2 mkm.

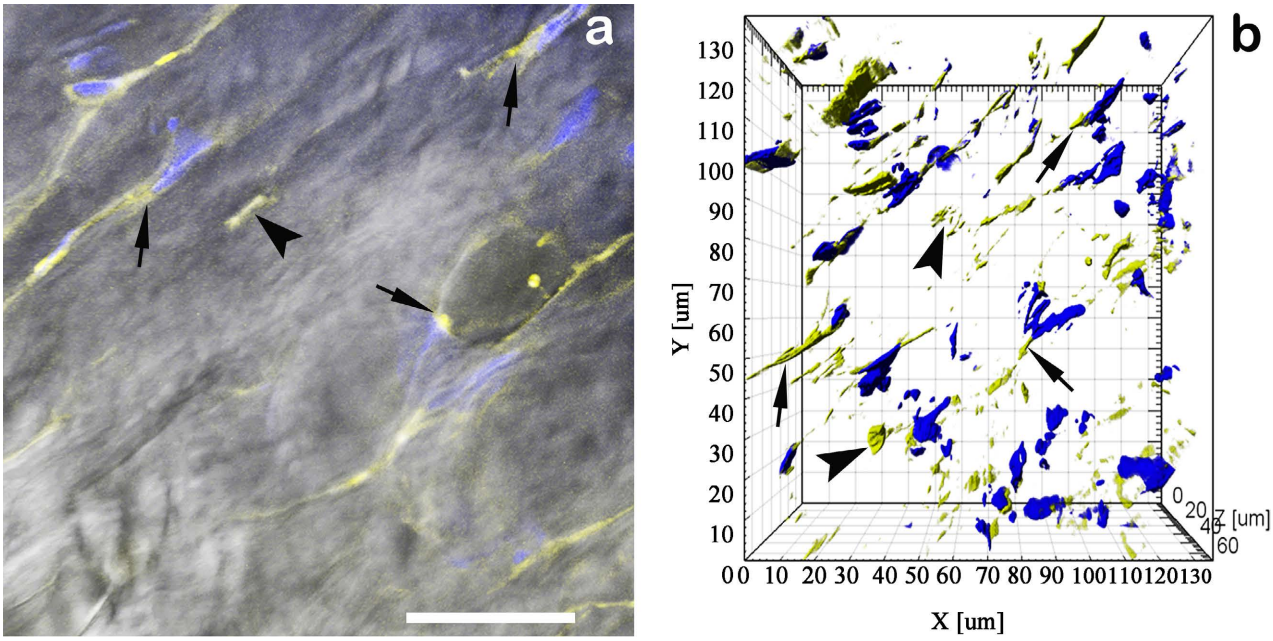

Figure 2. Intracellular and extracellular arrangement of neutral fats in the thickness of the lenticule anterior surface. Keratocyte nuclei stained with DAPI (blue), lipid droplets - Nile Red (yellow). Confocal microscopy. Transmitted light mode (a) and 3D-reconstruction (b) of secondary lenticule. Wide arrow: extracellular of neutral fats; narrow arrows: intracellular of neutral fats. Scale bar: (a) 20 mkm.

4.3. Posterior Surface Assessment

The ultrastructural organization of the lenticule posterior has a high degree of similarity with the lenticule after SMILE surgery [16] [17]. Scanning electron microscopy showed that the posterior surface had an ordered, ribbed surface with multiple needle structures 1.2 μm wide, 5 - 7 μm long, extending into the lenticular stroma. The lower section of such needle formations was braided with thinner threads forming a network structure (**Figure 3(a)**). Apparently, these structures were bundles of collagen fibers and microfibrils, torn off during the mechanical isolation of the lenticule (**Figure 3(b)**). In addition, ordered crater-shaped depressions, about 7 μm in size with clear edges, which were formed, apparently, as a result of the photodestructive effect of a femtosecond laser on the corneal tissue, were revealed on the surface of the lenticules (**Figure 3(c)**, **Figure 3(d)**).

Transmission electron microscopy revealed that the bulk of the intercellular matrix of the lenticular stroma was represented by correctly oriented layers of collagen fibers. In the thickness of the stroma, among the collagen bundles, individual keratocytes located along the plane of the lenticule were detected. Counting the number of nuclei on preparations stained with DNA-specific dye DAPI indicates that there were 37 (33; 40) nuclei in 1×10^6 mm^3 of the lenticular stroma.

Along the plane of the posterior surface of the lenticule, cells with varying degrees of degenerative changes were detected. These keratocytes had a deformed nucleus, large-lobed or strongly elongated with uneven contours, which contained a large nucleolus and finely dispersed chromatin, having the form of a

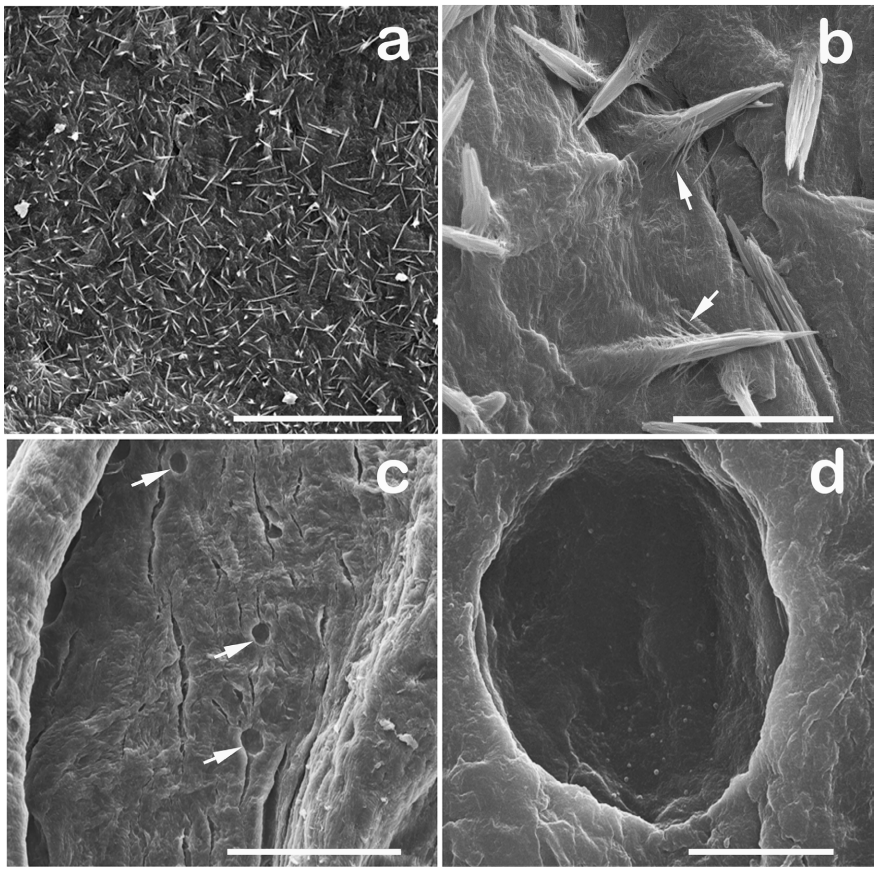

Figure 3. Scanning electron microscopy of the posterior lenticule surface. (a) posterior surface of lenticule with a large number of protruded collagen fibers; (b) collagen fibers with high magnification (thin collagen fibers pointed with arrows); (c) crater-shaped pit (pointed with arrows), probably formed as result action of femtosecond laser on cornea tissue; (d) pit on the surface of lenticule at high magnification. Scale bars: (a) 100 mkm; (b) 5 mkm; (c) 200 mkm; (d) 10 mkm.

finely granular osmiophilic matrix. In most of these cells, the cytoplasm remained structured, in which numerous small vesicles with different electron densities were found forming clusters in the recesses of the nucleus, which suggested the possibility of reversibility of the detected changes (**Figure 4(a)**). Other keratocytes had an enlightened cytoplasm that did not contain structured organelles, which corresponded to the picture of balloon dystrophy, which was irreversible [18] (**Figure 4(b)**). In addition, there were single dead cells, but their morphological signs were different. In some cells, the nucleus and individual sections of the cytoplasm were strongly fragmented into light and osmiophilic bodies surrounded by a membrane (**Figure 4(c)**), which was characteristic of death by apoptosis [19]. In other cells, the nucleus with finely granular chromatin was preserved, but the cytoplasm was destructured, and the cell membrane was absent for a long time (**Figure 4(g)**), which was a sign of colliquational cell necrosis [20].

There were keratocytes without visible pathology. Selective staining of preparations with phalloidin labeled with FITC showed that actin myofilaments are

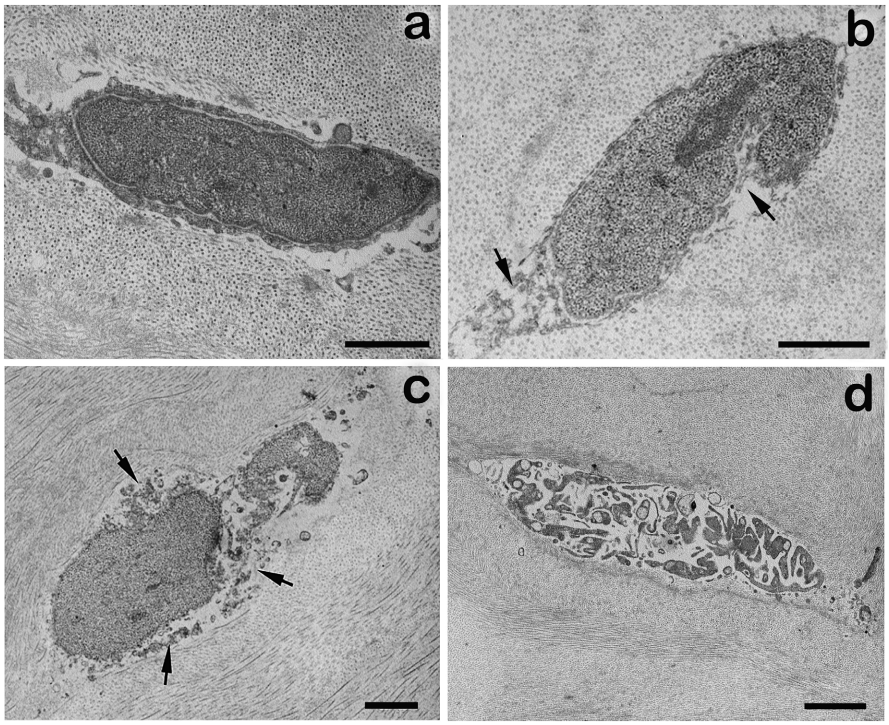

Figure 4. Degenerative changes of keratocytes in the lenticule. (a) keratocyte with intact contours of nucleus and with high osmiophilicity; ((b), (c)) keratocyte with local degenerative changes (pointed with arrows), with nuclear fragmentation and cytoplasm clarification; (d) keratocyte at final stage of degeneration. Scale bars: ((a), (c)) 1 mkm; ((b), (d)) 2 mkm.

one of the common components of the keratocyte cytoskeleton (**Figure 5(a)**). It is important to note that they are detected not only in the cytoplasm of keratocytes, but also in the extracellular space (**Figure 5(b)**, **Figure 5(c)**).

5. Discussion

Previous studies have evaluated lenticules extracted during SMILE surgery [16] [17]. This study is the first case of assessing the lenticule extracted during the correction of residual myopia 12 - 18 months after the SMILE surgery. It became possible, because the correction was carried out by a new method, which is the closest prototype of the sub-cap-lenticule-extraction (Sub-CAP-LE) method [21]. The basic principle of this surgery is the formation and removal of a new lenticule with the parameters calculated depending on the degree of residual myopia, which varied from −3.0 to −0.5 D. During surgery, the lenticule posterior surface and side cut incisions are formed similarly to the SMILE surgery. First, femtosecond laser exposure is accompanied by the formation of an acoustic shock wave, the release of thermal energy and the formation of cavitation bubbles, which underlies the photodestruction of the corneal stroma [22]. The lenticule anterior surface is distinguished only with the use of a Geuder spatula, since there is no dense fusion in this plane. After that the mechanical separation of the tissue is carried out at the level of the lenticule posterior surface and side

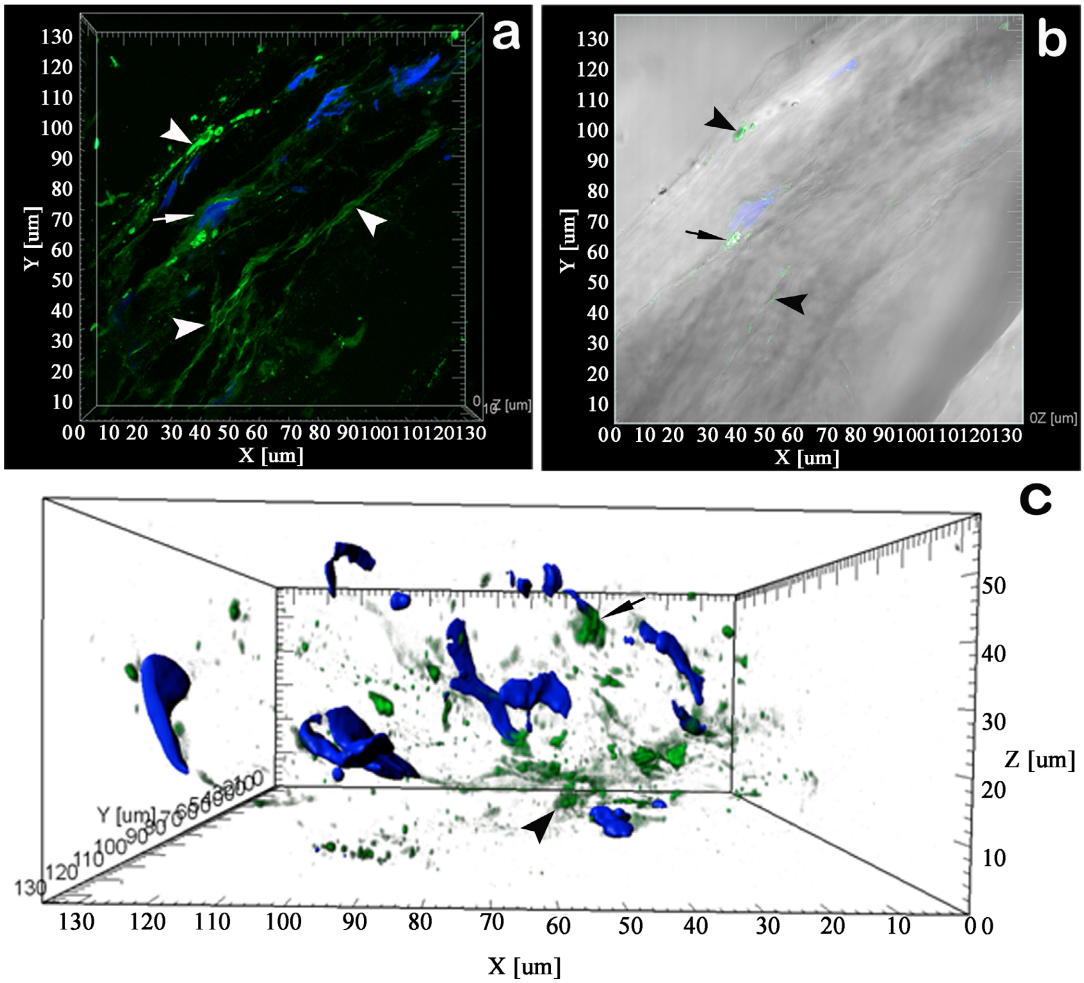

Figure 5. Intracellular and extracellular arrangement of actin microfilaments in lenticule. Staining for F-actin (FITC phalloidin, green) and for nuclei (DAPI, blue); laser confocal microscopy. ((a), (c)) 3D-reconstructions of lenticule fragment; (b) transmitted light mode of Z-stacks-3D-reconstructions of figure "a"; narrow arrows: intracellular of actin microfilaments; wide arrow: extracellular of actin microfilaments.

cut incisions. After that the lenticule is removed.

The anterior surface of a new lenticule is essentially the intrastromal space of the SMILE surgery performed earlier. Therefore, an ultrastructural assessment of this particular lenticule surface is of the greatest interest in revealing the mechanisms of corneal wound healing after SMILE. The biological mechanisms underlying the damage and corneal wound healing are a key factor determining the course of the postoperative period, the refractive effect and visual acuity of patients.

The indentations (7 - 10 μm) revealed in the study on the lenticule posterior surface are most likely the femtosecond laser exposure result and caused by the formation of cavitation bubbles, which is consistent with the work of a number of authors, whereas the numerous needle structures found on the surface of the lenticule are tissue bridges and undoubtedly formed as a result of lenticule mechanical separation from the corneal stroma [23]. These structures, judging by their size and localization, are mechanically torn collagen microfibrils and fibers

that carry out intrastromal connections between parallel collagen plates.

According to our data, keratocytes with reversible and irreversible degenerative changes are detected with the lenticule posterior surface, and some keratocytes undergo necrosis. It is possible as it is caused by local exposure to femtosecond laser energy at photodestruction of the corneal stroma along the line of lenticular separation. At the same time, the products of cell decay inevitably cause an inflammatory reaction, which in the cornea has features associated with the uniformity of cellular composition and the absence of blood vessels. Studies conducted by Teruo Nishida and Kaarina Tervo on a rabbit model have shown that the healing of corneal tissue after removal of the lenticule is accompanied by a cascade of biochemical reactions, including activation of cytokines and growth factors, which are released from the damaged epithelium and trigger apoptosis of stromal keratocytes, followed by their proliferation and migration to restore the damaged stroma. This is indicated by the moderate formation of TUNEL-positive, Ki67-positive keratocytes, CD 11b antigens [24].

In this study, it was shown that keratocytes containing F-actin in the cytoplasm are present on the posterior surface of the lenticule. This fact has a double meaning. First of all, it indicates the ability of keratocytes to produce F-actin and, probably, secrete it into the intercellular space, taking part in the regulation of actin-mediated apoptosis [25]. A number of authors point to the possibility of actin release from viable intact cells, while this protein can remain bound to the outer surface of the plasma membrane or exit into the extracellular medium, where it can form large aggregates [26] [27]. The role of extracellular actin in health and disease is actively discussed [28].

In addition, the accumulation of F-actin in the cytoplasm of keratocytes indicates the differentiation of these cells into myofibroblasts. It is known that keratocytes in a rarefied medium begin to transform into the so-called "repair phenotype" and into myofibroblasts that actively secrete elements of the extracellular matrix [29]. The importance of this fact is confirmed by many authors who believe that in the postoperative period, the balance between apoptosis of keratocytes and the development of myofibroblasts may be a critical factor determining the development of fibrosis and characteristic haze opacities [30].

In this study, significant differences have been found between the posterior and anterior surfaces. The anterior lenticule surface is smooth, with a weakly pronounced relief of collagen fibers that retain their integrity and are completely immersed in the lenticule stroma. This indicates that after SMILE, 12 - 18 months after surgery, at the level of the intrastromal space there is no restoration of collagen fibers original structure, no adhesion between the corneal flap and the corneal bed, no keratocytes modified by the type of myofibroblasts (containing actin myofilaments). However, neutral fats are detected perinuclearly in the cell processes and extracellular matrix, which are not detected on the posterior surface of the lenticule. It can be assumed that the appearance of fat inclusions is due to the transition of keratocytes to economical fat metabolism to re-

store their functional activity and represents a structural trace of the adaptation of cells to exist in the conditions prevailing in the corneal stroma after the first SMILE surgery [31].

The residual myopia correction technology after first SMILE surgery is based on the formation and extraction of a new lenticule. Its anterior surface is the intrastromal space after SMILE, and the posterior surface is formed as a result of femtosecond photodestruction of the cornea and mechanical separation of tissue bridges.

Structural changes in the posterior surface are characterized by changes similar to the lenticule study results during the first SMILE surgery. These are ruptures of collagen fibers on the surface of the lenticule, degenerative changes, and presence of keratocytes and F-actin in cytoplasm.

On the anterior surface of the lenticule, collagen fibers are immersed in the stroma, there is no complete structure restoration of collagen fibers, which explains the lack of dense fusion of the anterior and posterior surfaces of the intrastromal space even in the long-term period after surgery. There are no degenerative changes of keratocytes on the anterior lenticule surface, that is, their changes with SMILE are reversible in most cases. It is possible that when corneal homeostasis is restored, fat metabolism is being activated, but further studies are required to prove this.

The presented results prove the high efficiency and safety of SMILE.

The limitation of this study is the use of certain molecular markers and reagents, which do not allow a wider disclosure of the mechanisms of corneal wound healing after SMILE surgery.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Ratkay-Traub, I., Juhasz, T., Horvath, C., Suarez, C., Kiss, K., Ferincz, I. and Kurtz, R. (2001) Ultra-Short Pulse (Femtosecond) Laser Surgery: Initial Use in LASIK Flap Creation. *Ophthalmology Clinics of North America*, **14**, 347-355.

[2] Sekundo, W., Kunert, K., Russmann, C., Gille, A., Bissmann, W., Stobrawa, G., Sticker, M., Bischoff, M. and Blum, M. (2008) First Efficacy and Safety Study of Femtosecond Lenticule Extraction for the Correction of Myopia: Six-Month Results. *Journal of Cataract & Refractive Surgery*, **34**, 1513-1520. https://doi.org/10.1016/j.jcrs.2008.05.033

[3] Blum, M., Täubig, K., Gruhn, C., Sekundo, W. and Kunert, K.S. (2016) Five-Year Results of Small Incision Lenticule Extraction (ReLEx SMILE). *British Journal of Ophthalmology*, **100**, 1192-1195. https://doi.org/10.1136/bjophthalmol-2015-306822

[4] Guo, H., Hosseini-Moghaddam, S.M. and Hodge, W. (2019) Corneal Biomechanical Properties after SMILE versus FLEX, LASIK, LASEK, or PRK: A Systematic Review and Meta-Analysis. *BMC Ophthalmology*, **19**, Article No. 167.

https://doi.org/10.1186/s12886-019-1165-3

[5] Faussone Pellegrini, M.-S. and Popescu, L.M. (2011) Telocytes. *BioMol Concepts*, **2**, 481-489. https://doi.org/10.1515/BMC.2011.039

[6] Holmes, D.F., Gilpin, C.J., Baldock, C., Ziese, U., Koster, A.J. and Kadler, K.E. (2001) Corneal Collagen Fibril Structure in Three Dimensions: Structural Insights into Fibril Assembly, Mechanical Properties, and Tissue Organization. *Proceedings of the National Academy of Sciences of the United States of America*, **98**, 7307-7312. https://doi.org/10.1073/pnas.111150598

[7] Meek, K.M. and Knupp, C. (2015) Corneal Structure and Transparency. *Progress in Retinal and Eye Research*, **49**, 1-16. https://doi.org/10.1016/j.preteyeres.2015.07.001

[8] Tervo, K., Van Setten, G.B., Beuerman, R.W., Virtanen, I., Tarkkanen, A. and Tervo, T. (1991) Expression of Tenascin and Cellular Fibronectin in the Rabbit Cornea after Anterior Keratectomy. Immunohistochemical Study of Wound Healing Dynamics. *Investigative Ophthalmology & Visual Science*, **32**, 2912-2918.

[9] Nishida, T. (2012) The Role of Fibronectin in Corneal Wound Healing Explored by a Physician-Scientist. *Japanese Journal of Ophthalmology*, **56**, 417-431. https://doi.org/10.1007/s10384-012-0165-0

[10] Luft, N., Schumann, R., Dirisamer, M., Kook, D., Siedlecki, J., Wertheimer, C., Priglinger, S. and Mayer, J. (2018) Wound Healing, Inflammation, and Corneal Ultrastructure after SMILE and Femtosecond Laser-Assisted LASIK: A Human Ex Vivo Study. *Journal of Refractive Surgery*, **34**, 393-399. https://doi.org/10.3928/1081597X-20180425-02

[11] Vogel, A., Noack, J., Hüttman, G. and Paltauf, G. (2005) Mechanisms of Femtosecond Laser Nanosurgery of Cells and Tissues. *Applied Physics B*, **81**, 1015-1047. https://doi.org/10.1007/s00340-005-2036-6

[12] DelMonte, D.W. and Kim, T. (2011) Anatomy and Physiology of the Cornea. *Journal of Cataract & Refractive Surgery*, **37**, 588-598. https://doi.org/10.1016/j.jcrs.2010.12.037

[13] Birk, D.E., Fitch, J.M., Babiarz, J.P., Doane, K.J. and Linsenmayer, T.F. (1990) Collagen Fibrillogenesis *in Vitro*: Interaction of Types I and V Collagen Regulates Fibril Diameter. *Journal of Cell Science*, **95**, 649-657. https://doi.org/10.1242/jcs.95.4.649

[14] Dong, Z., Zhou, X., Wu, J., Zhang, Z., Li, T., Zhou, Z., Zhang, S. and Li, G. (2014) Small Incision Lenticule Extraction (SMILE) and Femtosecond Laser LASIK: Comparison of Corneal Wound Healing and Inflammation. *British Journal of Ophthalmology*, **98**, 263-269. https://doi.org/10.1136/bjophthalmol-2013-303415

[15] Pisarevskaya, O.V., Iureva, T.N., Shchuko, A.G., Frolova, T.N. and Khlebnikova, L.S. (2017) SMILE Post SMILE—A New Approach to the Correction of Residual Myopia. *Bulletin of the Volgograd State Medical University*, **61**, 108-110. (In Russian) https://doi.org/10.19163/1994-9480-2017-1(61)-108-110

[16] Kunert, K.S., Blum, M., Duncker, G.I.W., Sietmann, R. and Heichel, J. (2011) Surface Quality of Human Corneal Lenticules after Femtosecond Laser Surgery for Myopia Comparing Different Laser Parameters. *Graefe's Archive for Clinical and Experimental Ophthalmology*, **249**, 1417-1424. https://doi.org/10.1007/s00417-010-1578-4

[17] Ziebarth, N.M., Lorenzo, M.A., Chow, J., Cabot, F., Spooner, G.J., Dishler, J., Hjortdal, J.Ø. and Yoo, S.H. (2014) Surface Quality of Human Corneal Lenticules after SMILE Assessed Using Environmental Scanning Electron Microscopy. *Journal of Refractive Surgery*, **30**, 388-393. https://doi.org/10.3928/1081597X-20140513-01

[18] Strukov, A.I. and Serov, V.V. (2015) Pathological Anatomy: A Textbook. 6th Edi-

tion, GEOTAR-Media, Moscow. (In Russian)

[19] Green, D.R. and Reed, J.C. (1998) Mitochondria and Apoptosis. *Science*, **281**, 1309-1312. https://doi.org/10.1126/science.281.5381.1309

[20] Eingorn, A.G. (1976) Pathological Anatomy and Pathological Physiology. Medicine, Moscow. (In Russian)

[21] Donate, D. and Thaëron, R. (2015) Preliminary Evidence of Successful Enhancement After a Primary SMILE Procedure With the Sub-Cap-Lenticule-Extraction Technique. *Journal of Refractive Surgery*, **31**, 708-710. https://doi.org/10.3928/1081597X-20150928-04

[22] Juhasz, T., Djotyan, G., Loesel, F.H., *et al.* (2000) Applications of Femtosecond Lasers in Corneal Surgery. *Laser Physics*, **10**, 495-500.

[23] Sekundo, W. (2015) Small Incision Lenticule Extraction (SMILE): Principles, Techniques, Complication Management, and Future Concepts. Springer, Cham.

[24] Gourlay, C.W. and Ayscough, K.R. (2006) Actin-Induced Hyperactivation of the Ras Signaling Pathway Leads to Apoptosis in *Saccharomyces cerevisiae*. *Molecular and Cellular Biology*, **26**, 6487-6501. https://doi.org/10.1128/MCB.00117-06

[25] Müller, L.J., Pels, L. and Vrensen, G.F. (1995) Novel Aspects of the Ultrastructural Organization of Human Corneal Keratocytes. *Investigative Ophthalmology & Visual Science*, **36**, 2557-2567.

[26] Messmer, E.M., Meyer, P., Herwig, M.C., Loeffler, K.U., Schirra, F., Seitz, B., *et al.* (2013) Morphological and Immunohistochemical Changes after Corneal Cross-Linking. *Cornea*, **32**, 111-117. https://doi.org/10.1097/ICO.0b013e31824d701b

[27] Sudakov, N.P., Klimenkov, I.V., Byvaltsev, V.A., Nikiforov, S.B., Goldberg, O.A., Kalinin, A.A., *et al.* (2017) Extracellular Actin as a Factor in the Development of Degenerative Processes of Intervertebral Disc. *The New Armenian Medical Journal*, **11**, 20-26.

[28] Sudakov, N.P., Klimenkov, I.V., Byvaltsev, V.A., Nikiforov, S.B. and Konstantinov, Y.M. (2017) Extracellular Actin in Health and Disease. *Biochemistry*, **82**, 1-12. https://doi.org/10.1134/S0006297917010011

[29] Ljubimov, A.V. and Saghizadeh, M. (2015) Progress in Corneal Wound Healing. *Progress in Retinal and Eye Research*, **49**, 17-45. https://doi.org/10.1016/j.preteyeres.2015.07.002

[30] Ottani, V., Martini, D., Franchi, M., Ruggeri, A. and Raspanti, M. (2002) Hierarchical Structures in Fibrillar Collagens. *Micron*, **33**, 587-596. https://doi.org/10.1016/S0968-4328(02)00033-1

[31] Welte, M.A. and Gould, A.P. (1862) Lipid Droplet Functions beyond Energy Storage. *Biochimica et Biophysica Acta* (*BBA*)-*Molecular and Cell Biology of Lipids*, **1862**, 1260-1272. https://doi.org/10.1016/j.bbalip.2017.07.006