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Light and scanning electron microscopy of anterior lens capsule following different capsulorhexis techniques

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Aim — to assess the morphology of anterior lens capsules following circular capsulorhexis, for which capsulotomy was either manual or femtosecond (FS) laser produced. **Material and methods**. A total of 30 capsular samples obtained during phacoemulsification surgery were studied by light and scanning electron microscopy. All the samples were divided into 3 groups of 10 according to the type of capsulotomy: group 1 — manual, groups 2 and 3 — FS laser produced (with the laser set at high or low energy level, respectively). **Results**. In manual capsulorhexis, capsular disk edges were rather smooth all the way round. Rare notches and dents as well as fine burrs up to 4 μm long could only be seen under high magnification. In FS laser capsulotomy groups, under low magnification already, capsular disk edges appeared stepped, stratified, and notched (some notches were up to 20 μm deep). The said changes were more significant following low-energy laser treatment, while high-energy levels were notable for 'melted' disk edges. Regardless of the technique used, all the disks carried a zone of de-epithelization of different width along the free edge. **Conclusion**. The differences revealed may result from several factors: 1) less accurate focusing of the laser due to fine torsional eye movements and minimal shape changes of the applanated cornea; 2) peculiarities of FS laser-tissue interaction, particularly, cavitation bubbles that are formed during tissue evaporation and tend to merge, subsequently producing the incision and demarcation line. Moreover, photothermal effects of laser radiation on epithelial cells within the anterior lens capsule cannot be excluded.

Keywords: *hybrid phacoemulsification, femtosecond laser, lens capsule, capsulorhexis, light and scanning electron microscopy.*

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Today's developments in cataract treatment are mainly directed towards minimally invasive surgery. Besides ultrasound phacoemulsification (PE), which is widely used, new techniques are being introduced that imply anterior circular capsulorhexis and prefragmentation of the nucleus performed with a femtosecond (FS) laser [1-3]. By analogy from cardiovascular surgery, the term 'hybrid phacoemulsification' has been proposed [4]. FS laser light is near-infrared and has a wavelength of 1023 nm.

Potentially high quality of FS laser assisted capsulorhexis is considered one of the main benefits of hybrid PE. In order to minimize human factor in capsulorhexis quality assessment, such criteria as biomicroscopy findings and refractive results are used. It has been found that FS laser produces well-centered capsular openings of proper shape and location [5]. High elasticity of the capsular bag and even distribution of tensions allow stable central position of the intraocular lens (IOL) and, consequently, less refractive errors and optical aberrations [6, 7].

Several significant drawbacks of FS laser produced capsulorhexis have, however, been revealed. These include microtears visualized by scanning electron microscopy (SEM) of capsular fragments obtained during cataract surgery [5]. Similar results were reported by quite a number of authors, who also described cases of further enlargement of these microtears during surgery [8], a positive relationship between the cut surface irregularity and pulse energy level [9], and the presence of a 60-mm wide demarcation line along the edge of the resected capsule disk [10]. There was just one SEM-study, in which manual capsulorhexis appeared to be more harmful to capsule edges than laser treatment. Particularly, in manual technique, capsule edges were coarse and foliated, as opposed to laser-treated edges that were smooth and carried no tears [11].

Thus, using light and scanning electron microscopy, we aimed to obtain own data on the morphology of anterior lens capsules following circular capsulorhexis, for which capsulotomy was either manual or FS laser produced.

Material and methods

A total of 30 capsular tissue samples obtained during PE surgery were divided into 3 groups of 10 according to the type of capsulotomy: group 1 — manual, groups 2 and 3 — FS laser produced (using two different energy levels — 'low' and 'high', respectively). All surgical interventions were performed by the same surgeon. To achieve our ends, we first planned to collect more samples, but the homogeneity of the results enabled us to stop at 30, as mentioned.

For manual capsulorhexis, we used straight capsule forceps. All our movements were centripetal. In the two laser groups, VICTUS femtosecond laser (Technolas

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Perfect System, Germany) was used with the repetition rate set at 80 kHz, pulse duration at 230-550 fs, and wavelength at 1023 nm. Pulse energy levels of 5200 and 7000 nJ were specified as low and high, respectively. Spot spacing for the laser was 5 μm laterally and 2 μm in depth. The rigid interface diameter was 10.8 mm, curvature – 8.3 mm. Real-time guidance of the procedure was performed with an integrated OCT. Patient interface consisted of a tube, suction ring, applanation lens, and liquid-immersion solution between the lens and the cornea.

Immediately after retrieval, 5 samples from each group were immersed into a 10% neutral buffered formalin solution and then dehydrated through ascending ethanol concentrations. Romanovsky-Giemsa or hematoxylin and eosin-stained samples were covered with *Canadian balm*. Full-thickness slides thus obtained were studied under Leica DM2500 light microscope and imaged with Leica DFC320 (×25–1300) digital camera. For morphometric analysis of the images ImageScope Color software was applied.

The remaining 5 samples from each group underwent low-vacuum scanning electron microscopy on EVO LS10 machine (Carl Zeiss, Germany) at accelerating voltage of 20 kV. For that, non-fixed capsule disks were placed horizontally on the stage of the microscope with the epithelial surface facing up.

Results and discussion

Anterior lens capsules following manual capsulorhexis

At light miscroscopy of flat glass slides, capsule disk edges appeared rather smooth all the way round. No tears or burrs were found. In the periphery of the underside of each disk, there was a de-epithelized zone of 11.9±3.8 μm wide, which is comparable to the average diameter of capsular epitheliocyte (**Fig. 1**).

Under higher magnification, the epithelial lining demonstrated a waveform tear-off line that followed either cell, or nuclei contours depending on whether or not the cells were damaged. The possibility of transcellular tearing is also suggested by the fact that the average area of marginal cells was found to be less than that of more centrally-located cells (145±24.7 μm^2 and 188±25.4 μm^2, respectively), although their nuclei were of similar shape and size (38.8±5.5 μm^2 and 46.6±9.3 μm^2, respectively). Some portions of the lining, however, reached the edge of the disk and even rolled back a short distance (**Fig. 2**). It is possible that different tearing patterns are due to different levels of adhesion among epithelial cells and changes in their functional status. One can also suppose that the de-epithelized zone on the resected capsule disk is mirrored by the free edge of the remaining lens capsule. Two

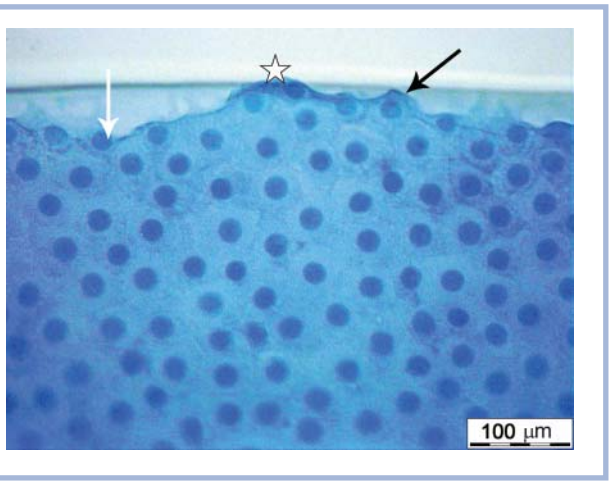

Fig. 2. **Anterior lens capsule following manual capsulorhexis: tearing of lens epithelium. The tear-off line goes mostly along cell borders (black arrow), though some cell walls are also damaged (white arrow) and folded (star). Flat glass slide. Romanovsky-Giemsa stain.**

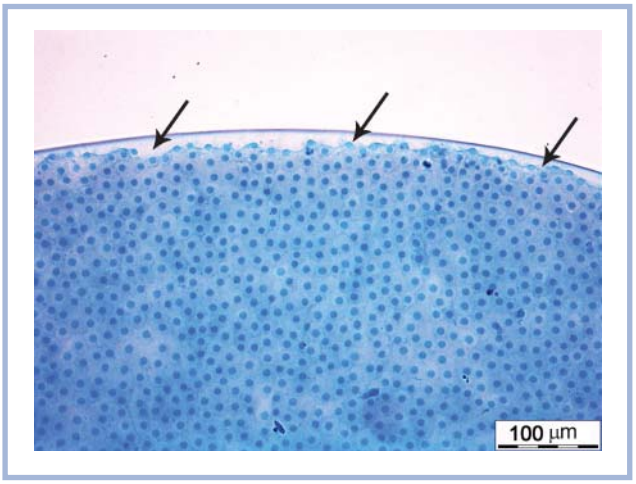

Fig. 1. **Anterior lens capsule following manual capsulorhexis: smooth capsule disk edges. A narrow zone of de-epithelization can be seen along the free edge of the capsule (arrows). Flat glass slide. Romanovsky-Giemsa stain.**

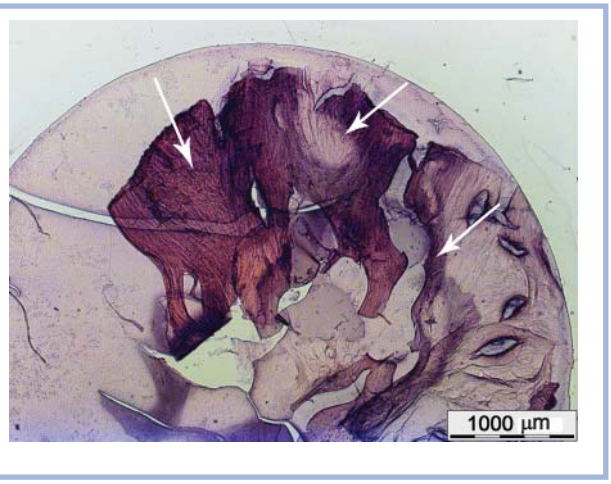

Fig. 3. **Anterior lens capsule following manual capsulorhexis: cortical fiber cells removed with the capsule (arrows) Flat glass slide. Hematoxylin and eosin stain.**

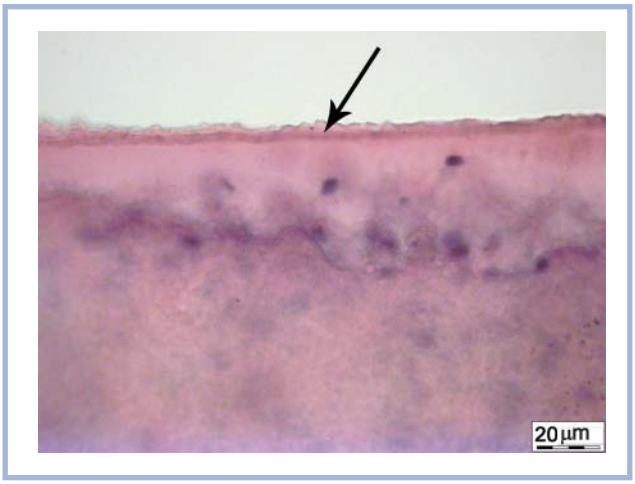

Fig. 4. **Anterior lens capsule following manual capsulorhexis: under higher magnification, the cut line looks ragged (arrow). Flat glass slide. Hematoxylin and eosin stain.**

Fig. 5. **SEM-image of an anterior lens capsule following manual capsulorhexis: along the free edge of the capsule disk, there is a bounded de-epithelized zone (in between yellow arrows), whose contour is irregular (white arrow) due to small protuberances and notches.**

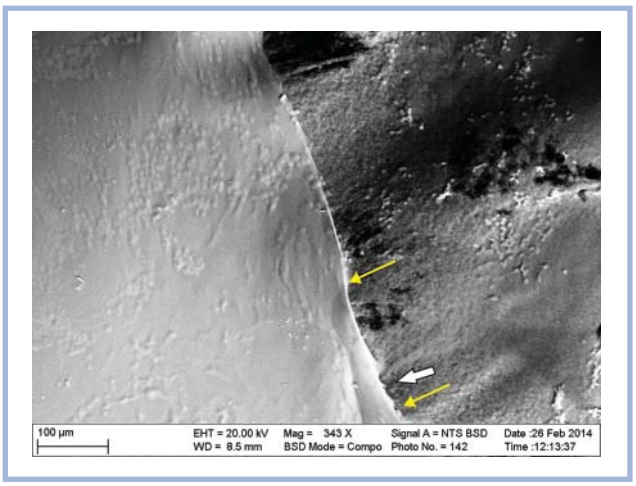

Fig. 6. **SEM-image of an anterior lens capsule following manual capsulorhexis: against the background of generally even cut surface of the disk edge (in between the white and the black arrows) a single burr is seen (white arrow).**

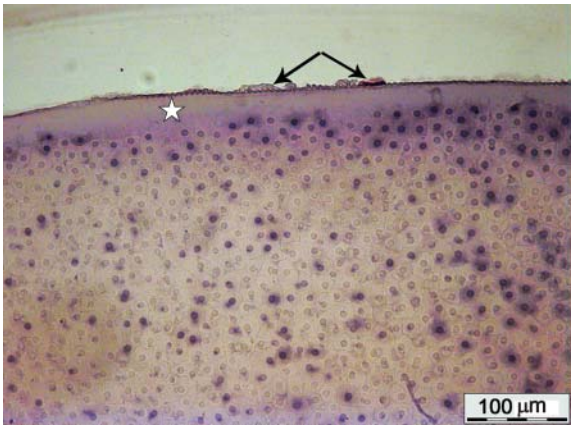

Fig. 7. **Anterior lens capsule following low-energy femtosecond laser produced capsulotomy: capsule surface along the free edge of the disk is ragged (arrows), while the de-epithelized zone is wider than that in manual capsulorhexis (star). Flat glass slide. H**ematoxylin and eosin stain**.**

samples from this group additionally had some cortical fiber cells attached to their underside (**Fig. 3**), which probably indicates a particular type of cataract in these patients. As for the cut surface of the disks, it looked ragged, each burr being not longer than 2-4 µm (**Fig. 4**).

At scanning electron microscopy, the zone of de-epithelization was never complete. Free edges of the disks carried some burrs and dents (**Fig. 5**). The cut surface showed a laminated relief. The epithelial lining was irregular all the way round and carried some notches (**Fig. 6**).

Anterior lens capsules following low-energy femtosecond laser produced capsulorhexis

At light microscopy, free edges of capsule disks, though generally clean, showed some roughness and quite a number of protuberances (**Fig. 7, 8**). The zone of de-epithelization was 1.5 times wider than that in the first group and averaged 15.9±3.73 µm. Marginal epithelial cells had thicker-than-usual cell walls adjacent to the nuclei. A decrease in the average area of these cells as well as the average area of their nuclei (down to 140.1±30 and 21.2±4.7 µm^2, respectively) was mostly associated with kariopyknosis, which indicates possible thermal effect of femtosecond laser radiation. Epithelial cells that layed more proximally were comparatively larger and had bigger nuclei (148.4±36.3 and 26±4.1 µm^2, respectively), though not as big as those from the manual capsulorhexis group, which may be due to laser-induced contraction of the capsule.

SEM-images (x1300) of laser-treated lens capsules showed evident de-epithelization of their underside (up

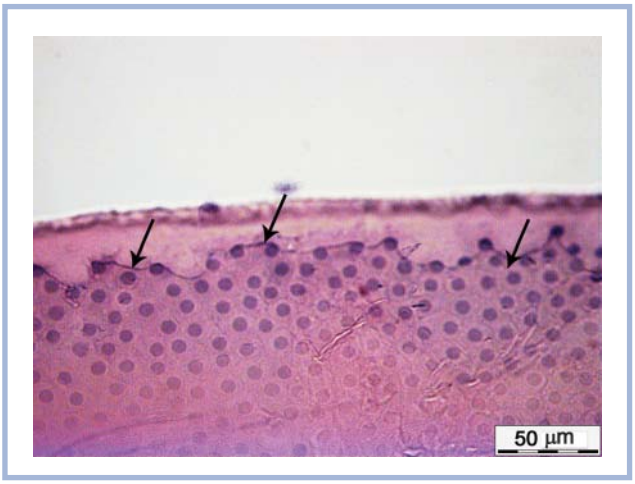

Fig. 8. **Anterior lens capsule following low-energy femtosecond laser produced capsulotomy: under higher magnification, one can see that the edge of the capsule disk is irregular, cell walls of marginal epitheliocytes (arrows) are less dense than usual, and the average area of their nuclei is decreased. Flat glass slide. Hematoxylin and eosin stain.**

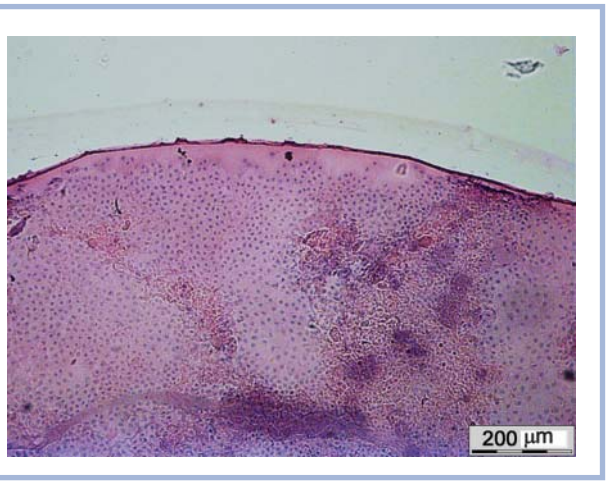

Fig. 9. **SEM-image of an anterior lens capsule following low-energy femtosecond laser produced capsulotomy: irregular capsule disk edge, unevenly widened acellular zone, and smaller-than-usual marginal epitheliocytes (arrows).**

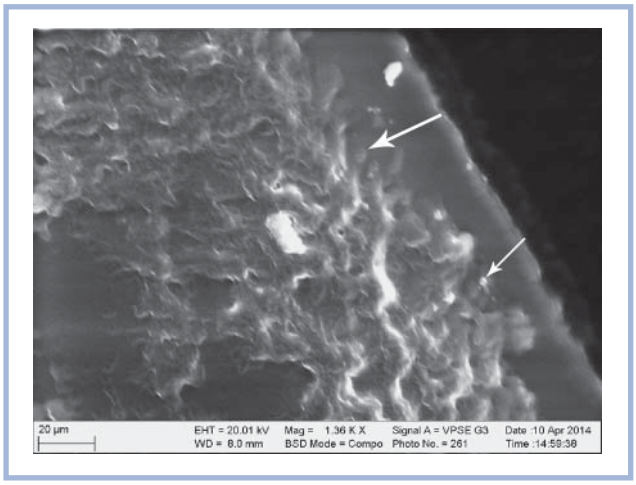

Fig. 10. **Anterior lens capsule following high-energy femtosecond laser produced capsulotomy: some roughness can be seen along the free edge of the capsule disk. Flat glass slide. Hematoxylin and eosin stain.**

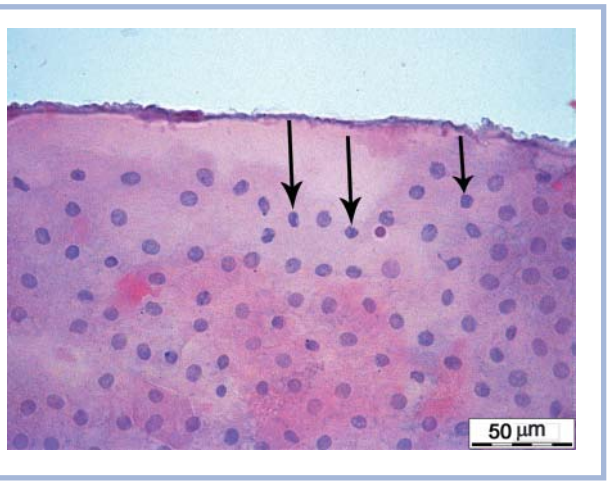

Fig. 11. **Anterior lens capsule following high-energy femtosecond laser produced capsulotomy: under higher magnification, there is an evident alternation of areas with regular and irregular cell arrangement; marginal epithelial cells are polymorphic and decreased in size. Their nuclei are pyknotic (arrows). Flat glass slide. Hematoxylin and eosin stain.**

to 35 µm wide zones), irregular tear-off line of the epithelial lining, and smaller-than-average marginal epithelial cells (**Fig. 9**).

Anterior lens capsules following high-energy femtosecond laser produced capsulorhexis

Free edges of capsule disks carried no gross changes, though some protuberances and notches were still present demonstrating a slightly stronger stain uptake than most capsular structures (**Fig. 10**). The de-epithelized zone was even wider in this group — up to 35.6±14.8 µm. Along its margin, cell nuclei were packed and deformed over a larger distance as compared to other groups (**Fig. 11**). The average area of marginal epitheliocytes was as small as 126±24.7 µm^2 (and that of their nuclei — 23.2±5.4 µm^2). Further towards the center, the average cell size rapidly increased up to 183.4±24.8 µm^2 (35.6±12.5 µm^2 for the nuclei), but did not reach the values from the manual capsulorhexis group.

Under low magnification of the scanning electron microscope, free edges of the disks were generally smooth and carried only shallow hollows and/or strata (**Fig. 12**).

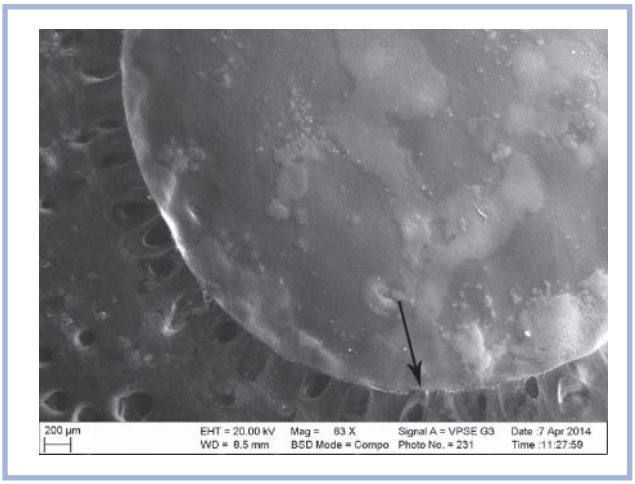

Fig. 12. **SEM-image of an anterior lens capsule following high-energy femtosecond laser produced capsulotomy: here and there, the free edge of the capsule disk is notched (arrow).**

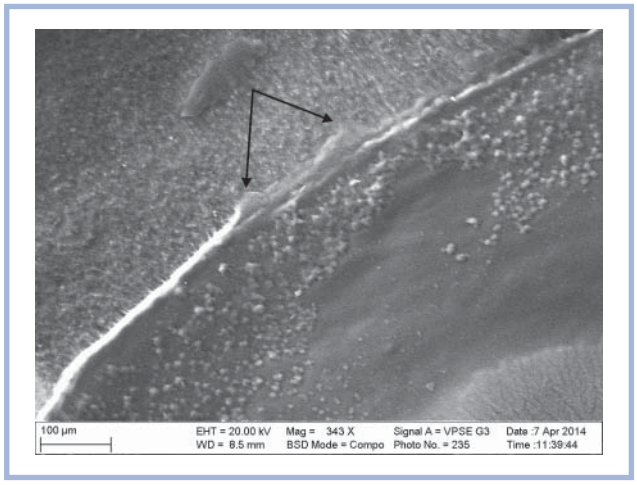

Fig. 13. **SEM-image of an anterior lens capsule following high-energy femtosecond laser produced capsulotomy: under higher magnification, the end face of the capsule looks 'melted' and partially foliated (arrows).**

Under higher magnification, these strata turned out to be capsular layers rolling back over the underside of the disks. Over a large area, however, disks' end faces looked 'melted' and did not show significant foliation (**Fig. 13**).

Conclusion

By comparison of light and scanning electron microscopy findings of lens capsular fragments obtained during phacoemulsification cataract surgery with either manual, or femtosecond laser produced capsulotomy, some stark differences have been revealed, as expected.

First to be noted is that, in manual capsulorhexis, capsule disk edges were rather smooth all the way round. Rare notches and dents as well as fine burrs up to 4 µm long could only be seen under high magnification. In FS laser capsulotomy groups, under low magnification already, end faces of the disks appeared stepped, stratified, and notched (some notches were up to 20 µm deep). The said changes were more significant following low-energy laser treatment, while high-energy levels were notable for 'melted' disk edges.

We have also established that, regardless of capsulorhexis technique, there is always a zone of de-epithelization along the free edge of the capsule disks. In the manual capsolotomy group, this zone was only 11.9±3.8 µm wide (which is comparable to the average diameter of epitheliocyte) and incomplete, or irregular (some cells were still in their places). The tear-off line resembled a half of a zipper and apperently was congruent to the profile of the remaining lens capsule. In the FS laser groups, as the energy increased, the de-epithelized zone became even wider (15.9±3.73 and 35.6±14.8 µm, respectively). Previous studies described a so called *demarcation line* (however, the term *zone* would be more appropriate) of about 60 µm wide that was due to laser-induced destruction and desquamation of epithelial cells [10]. It is possible that such a zone triggers re-epithelization process, which involves cell proliferation in germinative (equatorial) region and is associated with higher risk of secondary cataract. Moreover, some findings (particularly, a decrease in the average area of epithelial cells along the demarcation zone, tortuosity of cell borders, kariopyknosis, and ‹melted› appearance of capsule edges) indicate that FS laser may have not only a photodestructive, but also a photothermal effect on the anterior lens capsule and its epithelium.

We agree with conclusions made by foreign authors regarding the reasons for varying ‹quality› of capsulorhexis (manual or FS laser assissted). The following factors may be responsible:

1) less accurate focusing of the laser due to fine torsional eye movements and minimal shape changes of the applanated cornea during docking;

2) peculiarities of FS laser-tissue interaction, particularly, cavitation bubbles that are formed during tissue evaporation and tend to merge, subsequently producing the incision and demarcation zone (the so called photodestructive effect).

Also, taking into account epitheliocytes contraction in the area and kariopyknosis, one cannot exclude photothermal effects of laser radiation on cellular structures within the anterior lens capsule.

Author contributions:
Study conception and design — K.A., A.F., I.N.
Acquisition and handling of data — K.A.
Statistical analysis of data — A.F., I.N.
Drafting of manuscript — K.A.
Critical revision — A.F., I.N.

The authors declare no conflict of interests.

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