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Biomechanical properties of the anterior lens capsule after manual and femtolaser capsulotomy

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Purpose — to comparatively evaluate the mechanical stability of the edge of central fragment of anterior lens capsule after manual and femtolaser capsulotomy. **Material and methods.** The mechanical tests were performed on the central fragments of the anterior lens capsules obtained intraoperatively after manual and femtolaser capsulotomy (15 and 13 samples, respectively). The conditions of the developed method of mechanical testing are as close to *in vivo* as possible. The method allows evaluation of the mechanical response mainly from the edge of the sample, reception of the averaged response from the four edges of the capsule, and almost eliminates the effect of additional edge notches in samples obtained by manual capsulotomy. **Results.** After manual capsulotomy, the maximum force and elongation of the anterior capsule sample at maximum tensile strength were significantly higher than similar characteristics of the samples after femtolaser capsulotomy. **Conclusion.** The obtained results correspond with the morphological studies of capsule edge structure after manual and femtolaser capsulotomy. The edge of the anterior capsule after femtolaser capsulotomy has form, in rough approximation, close to one of a postage stamp perforation, which is the consequence of micro-irregularities (microfractures) in the areas irradiated by pulsed laser, and wider area of deepithelization compared to the manual technique.

Keywords: *lens capsule, anterior capsulotomy, biomechanics of anterior lens capsule, femtosecond laser radiation.*

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Crystalline lens capsule is an outer membrane, which is fairly tight and elastic — properties that largely define its anatomical and functional characteristics. Discretion between the two parts of the membrane — anterior and posterior — is motivated by not only topography, but also morphology. Thickness of the anterior lens capsule exceeds the posterior counterpart by several times and lies in the range of 4—24 μm, which in turn significantly improves its mechanical strength properties (especially of its central portion). According to classical understanding, in terms of biomechanical studies crystalline lens should be regarded as having innate functionality for changing its shape (i.e. deformation) that provides capability to strengthen or weaken overall optical power of the eye — the process known as accommodation [1].

Modern trends in surgery of crystalline lens (phacosurgery) help expand these views by outlining another research vector in lens biomechanics — evaluation of mechanical properties of the lens capsule. Anterior capsulotomy (continuous curvilinear capsulorhexis; CCC) is an important technological step in microinvasive phacosurgery; its quality greatly affects whether the following surgical steps (core fragmentation, removal of lens mass, implantation of intraocular lens — IOL) will be performed correctly and adequately. Among other characteristics of anterior capsulotomy (diameter, centering), biomechanical properties of its edge have been emphasized recently in the context of its resistance to intracapsular manipulations and the positional stability of IOL.

First experimental studies on biomechanical properties of the lens capsule were aimed at advancing fundamental knowledge, not at practical application. As such, in a series of experimental studies R. Fisher et al. studied elastic constants (authors' own terminology) of cadaver (i.e. obtained *ex vivo*) lens capsule from human and animal eyes [2—4]. For comparison, the study used vulcanized rubber similar to lens capsule in thickness. Values of Young's modulus (modulator of direct elasticity, N/mm^2 or MPa) — a physical quantity measuring the ability of a material to withstand positive/negative stretching when under elastic strain, defined as a ratio between stress and strain of the lens capsule or rubber membrane, differed significantly: in rubber membrane it decreased with stretching. Strain of the lens capsule (relative extension of the specimen calculated as displacement divided by initial length) before tearing was 25% (in rubber membrane — several orders more), while maximum mechanical stress (defined as a ratio of stretching force to unit of area in the given point of the section) before capsule tearing — 2.3±0.7 N/mm^2 (N is for newtons). According to those studies,

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anterior lens capsule should be viewed as a multilayer sheet of collagenous macromolecules wound in the form of superhelices which extend under stress.

Another (comparative) study [5] assessed biomechanical properties of Descemet's membrane and anterior lens capsule of cadaver eyes of cows, sows, rats and humans. In animal specimens, Descemet's membrane was less prone to strain than lens capsule, and in humans these structures showed similar mechanical properties.

Further research on biomechanical properties of the lens capsule was application-oriented – it dealt with age-related changes, significance of capsule biomechanics for the mechanism of lens deformation, and the effect of various capsulotomy methods used during phacosurgery on mechanical strength of the capsule.

S. Krag et al. [6] have analysed age dependency of biomechanical properties of human anterior capsule *ex vivo*. According to their studies, capsule thickness increases gradually until the age of 75 years, and then it starts to decrease slightly. Yearly age-related decrease of the biomechanical properties such as ultimate strain, ultimate tensile strength and ultimate elastic stiffness (Young's modulus) was 0.5, 1.0 and 0.9%, respectively. Elasticity of the lens capsule was shown to be the dominant factor in lens shape, while its age-related changes may have some significance for the development of presbyopia [7, 8].

As suggested above, biomechanical properties of the anterior lens capsule in terms of phacosurgery have significance due to the technical features of modern microinvasive techniques (particularly those that include such elements as capsulotomy, tampering in the capsular bag, IOL implantation), which may cause substantial deformation of the capsule. The present interest in this problem is associated with, among other reasons, practical application of femtosecond laser-assisted anterior capsulotomy. Unlike the manual technique, it is based on dissecting and tearing the capsule through a tunnel section using special instruments, ablation of the tissues is done before opening the fibrous membrane due to laser-induced formation and collapse of minute gas bubbles.

Essentially, the possible differences in the biomechanical parameters of the anterior capsule after manual or femtolaser capsulotomy are manifested in the structural features of capsule edge after these procedures [9–14]:

Anterior capsule edge after femtosecond laser-assisted capsulotomy is uneven, similar to postage-stamp perforations (rough approximation).

Electron microscope investigation showed that the anterior capsule edge had irregularities (microgrooves) after exposure to femto-second laser – traces of laser impulses.

When performing manual capsulorhexis, the profile of free capsule edge looks more even along the perimeter, and only high magnification reveals rare notches, small dents and "burrs" up to 4 μm long.

Regardless of the method, there is a variously prominent deepithelialization area along the free edge of the anterior capsule: after manual capsulotomy, the width of the cell-free area is similar to the diameter of one epithelial cell, while after femtolaser – the width of the borderline cell-free area increases in proportion to the power of femtosecond laser.

To assess biomechanical "stability" of the anterior capsule edge after various capsulotomy surgeries, cadaver porcine [15–18] and human eyes [19–22] were analyzed. Comparison of these studies presented in the literature review published earlier led to the following key conclusions [23]:

1. Assessment of biomechanical properties using human and porcine anterior capsule specimens obtained *ex vivo* as experimental models does not eliminate the possible effect of postmortem changes or anatomical features of animal capsule on the results of such studies.

2. Variability of the absolute measurements in different studies that characterise the resistance of anterior capsule against tearing (tearing strength, elasticity, extension) may be explained by a number of factors: the choice of experimental model, capsulotomy technique, method of specimen acquisition, properties of mechanical tests. Therefore, it is reasonable to utilize relative measurements acquired in the course of the present study for comparison of mechanical stability of the capsule edge after different capsulotomy techniques.

3. Considering the non-homogeneity of the results of different studies, comparative assessment of mechanical stability of human lens capsule after manual and femtolaser capsulotomy requires further investigation, in conditions as close to *in vivo* as possible.

4. Mechanical testing of the peripheral edge of capsulotomy opening implies that the remaining capsular bag space should be filled with some sort of viscous substance (gelatine, hyaluronic acid) and the capsule edge should be "wetted", which can all affect its mechanical stability.

As such, we proposed to assess biomechanical stability of the anterior capsule using central portion of human anterior capsule obtained during minimally invasive phaco surgery instead of the edge of capsular bag and its peripheral parts (as was done in most other studies) [23]. Theoretical premises for this approach are based on the fact that central and peripheral capsulotomy edges are congruent. Similar model was employed in one comparative study published later [24], which however did not include a detailed description of the process of preparing central portion of the capsule after manual and femtolaser capsulotomy for "breaking strength" tests. This factor, in our opinion, has essential significance in the matter, especially considering the thickness of the capsule and – above all – the difference in specimen configurations due to the presence of an additional notch (incision) on the sample edge after manual capsulotomy **(Fig. 1)**.

The purpose of the present study is to evaluate and compare biomechanical properties of anterior lens capsule after different capsulotomy techniques using a testing method with conditions as close to *in vivo* as possible.

Material and methods

The study involved 28 lens capsule specimens obtained during surgical treatment of cataract by curvilinear (5–5.5 mm in diameter) manual and femtosecond laser-assisted capsulotomy (15 and 13 specimens, respectively). All surgeries were performed by the same surgeon. The mechanical testing itself involved 8 specimens from each group, while all other specimens were used to refine the testing procedure. Initially, the study was planned to involve higher amount of anterior capsule specimens, but homogeneity of the results allowed analysis to be limited to the indicated sample size.

Manual capsulorhexis, after preliminary "notching" of the anterior capsule, employed capsule forceps with straight jaws and so-called centripetal movement. For femtosecond capsulotomy, VICTUS laser platform (Technolas Perfect Vision, Germany) was used. Infrared femtosecond laser was set up for 80 kHz frequency, 230–550 fs impulse length and 1023 nm wavelength. Energy level while performing capsulorhexis was 7000 nJ, spot separation was 5 μm, and layer separation – 2 μm. Surface diameter of the "hard" interface was 10.8 mm, curvature – 8.3 mm. Femtosecond laser-assisted capsulotomy was controlled in real time using OCT device integrated into the laser platform. Patient interface consisted of applanation lens, immersion solution between the lens and cornea, vacuum ring and tube. Specimens were put into saline solution, time intervals between sample acquisition and testing were 1.5–5 hours.

Patients were all between 55 and 60 years old. Exclusion criteria were: diabetes mellitus, pronounced subluxation of the crystalline lens, pseudoexfoliation syndrome and insufficient preoperative mydriasis.

Fig. 2 shows the algorithm of sample capsule testing (Russian patent no. 2018132897 for invention "method of evaluating biomechanical properties of the anterior lens capsule" by Avetisov K.S. et al. filed on 17.09.2018). The process of mechanical testing, observed with a microscope, involved anterior capsule specimens being suspended on a trapezium-shaped wire hook in such a way so that its bend fitted the specimen's diameter. When placing the specimens obtained by manual capsulotomy, the notch on the capsule edge was arranged perpendicularly to the bend on the hook. The upper end of the wire was then secured, free specimen edges were attached to the pneumatic clamps of INSTRON 3365 testing device (Germany); pressure of the pneumatic system was 2 atm. After balancing the position and load, monoaxial expansion of the specimen was performed at 2 mm/min speed until it teared (**see Fig. 1 – c, d**). The force was measured using a sensor with 10H±0.25% measurement range and linearity of ±0.25%, the signal from the sensor was registered at 10 Hz. Biomechanical properties of the anterior capsule edge were evaluated by extension under maximum stretching force; the results were recorded in a form of force-displacement graphs.

Results and discussion

Study algorithm involved several objectives. The first, as described above, was related to the need for testing conditions to be as close to *in vivo* as possible. To solve this, mechanical testing was performed on central portions of the anterior capsule that were removed intraoperatively, during microinvasive phaco surgery, as the result of manual and femtolaser capsulotomy. The testing was done in the next several hours after acquiring the specimens, which until then were preserved in a balanced physiological solution.

The second objective was to comply with potential standardization requirements for preparation of capsulotomy specimens obtained by different methods for use in mechanical testing: while the specimens from laser capsulotomy were close to round in shape, the specimens from manual capsulotomy had a notch (tear) on their edge due to initial local opening of the anterior capsule (**see Fig. 1**). Besides, it should be considered that the lower range limit of the sensitivity of mechanical sensors used in the testing was close to n$\cdot 10^{-2}$–10^{-5}H. Such sensitivity is sufficient for evaluation of biomechanical properties of most biological structures, including the mechanics of various macromolecules. Despite the sensors used in testing having some margin of sensitivity, consistent evaluation of biomechanical properties of the edge of a lens capsule fragment has certain technical difficulties. The first problems is that the sensor operating in the lower range of sensitivity may have lower signal-to-noise ratio. The second problem – initial inhomogeneity of the properties of the edge of a lens capsule fragment; it requires multiple measurements to accumulate statistical data, which is problematic due to physical dimensions of the object of measurement. The third problem – the geometry of the stretching force applied to disc-shaped object partially attached to the pneumatic clamps will produce strain primarily on the axis of stretching, which will hinder evaluation of biomechanical properties of its edges. Lastly, the fourth problem – additional tearing in the capsule that is inherent for manual capsulotomy should not be involved in the response of the specimen to applied stretching.

The suggested method of mechanical testing neutralizes these difficulties to a degree thanks to innovative specimen attachment technique that allows:

– evaluation of mechanical response of particularly the specimen›s edges;

– acquisition of average response from the four edges, which on the one part solves the problem of inhomogeneity, and on the other part – raises the force registered by the sensor to the more accurate range of sensitivity;

– practically eliminates the influence of the additional notch on specimen›s edge.

Results of the mechanical testing of anterior capsule specimens obtained after different capsulotomy techniques can be seen in **Table 1**. When testing manual capsulotomy specimens, the maximum stretching force and specimen

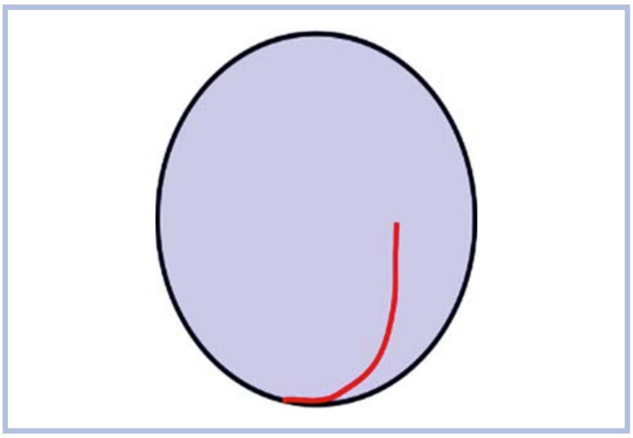

Fig. 1. **Schematic representation of central capsule fragment configuration after manual capsulotomy.**

The red line corresponds to the notch on the capsule edge.

extension under maximum stretching force were significantly higher than in specimens after femtolaser (median 0.136 N and 6.58 mm for manual, 0.055 N and 3.90 mm for femtolaser, respectively). This shows that capsule edge after femtolaser has lower mechanically durability. Central and peripheral capsulotomy edges are mirroring each other, which provides circumstantial basis for such conclusion to also be applied for the edge of preserved capsular bag. For clarity, see **Fig. 3** that shows force-displacement diagrams for anterior capsule specimens obtained by different capsulotomy methods. The resulting differences can be regarded as expected (to some extent), especially considering the listed above results of morphological studies that investigated structural changes of the capsule edge after manual and femtolaser capsulotomy. As mentioned above, anterior capsule edge after femtolaser capsulotomy has postage-stamp perforations (an approximation) due

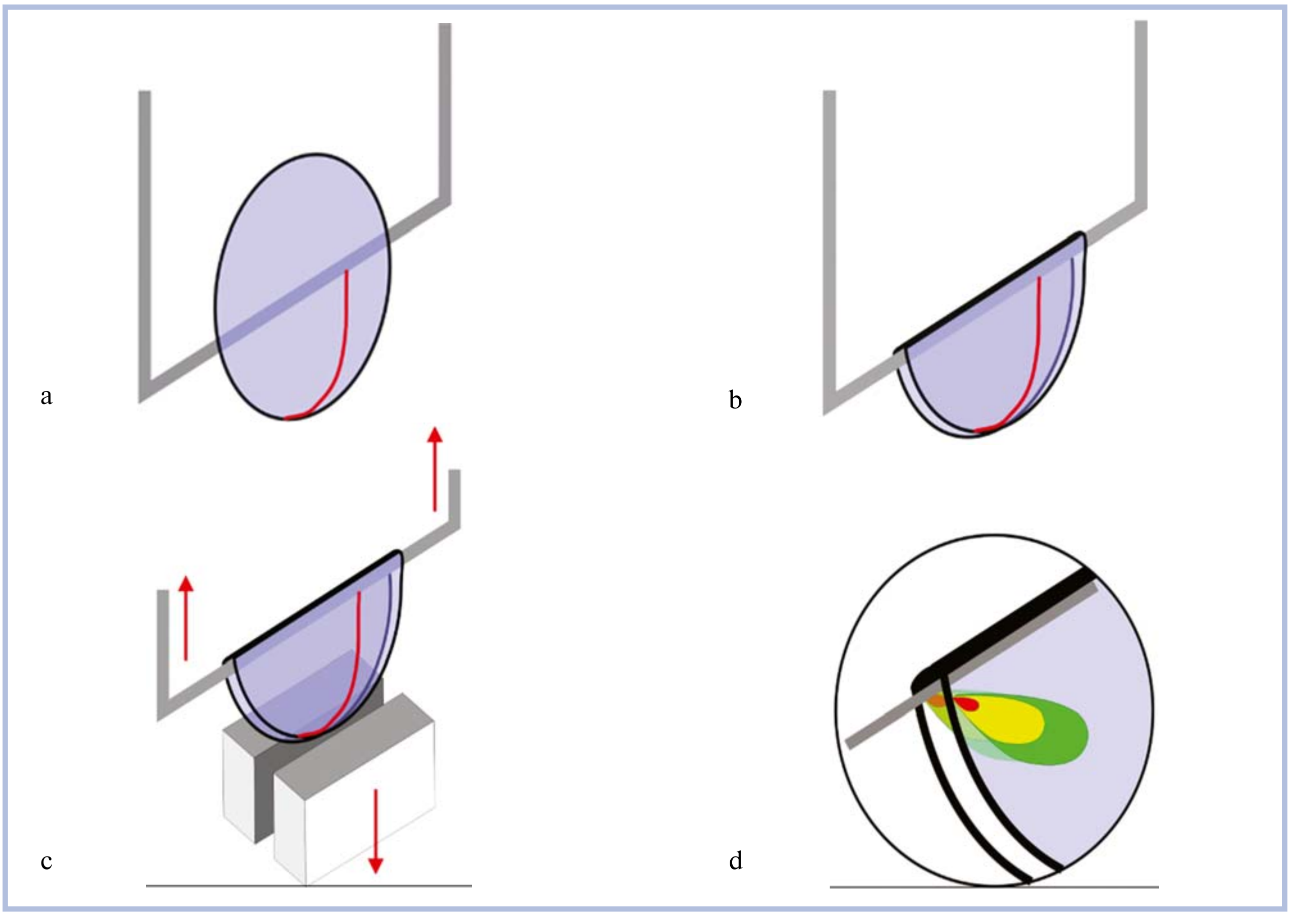

Fig. 2. **Algorithm of mechanical tests by the example of anterior capsule sample obtained after manual capsulotomy.**

a, b — the sample is fixed on a loop of wire: the fold corresponds to the sample diameter, notch on the capsule edge is perpendicular to the fold of the wire loop; c — free edges of the sample are held in pneumatic clamps of the testing device (marked with arrows are the directions of sample stretching); d — stress fields emerging during the testing of the sample (marked with arrow): simultaneous mechanical response from two edges is present on each of the two diametrical sides.

Results of mechanical tests performed on anterior lens capsule samples after different capsulotomy techniques

Parameter	Value	Technique	
		manual	laser
Maximum force, N	Median (min-max)	0,136 (0,037—0,660)	0,055 (0,048—0,091)
Extension under maximum stretching force, mm	Median (min-max)	6,58 (1,56—13,61)	3,90 (3,76—5,01)

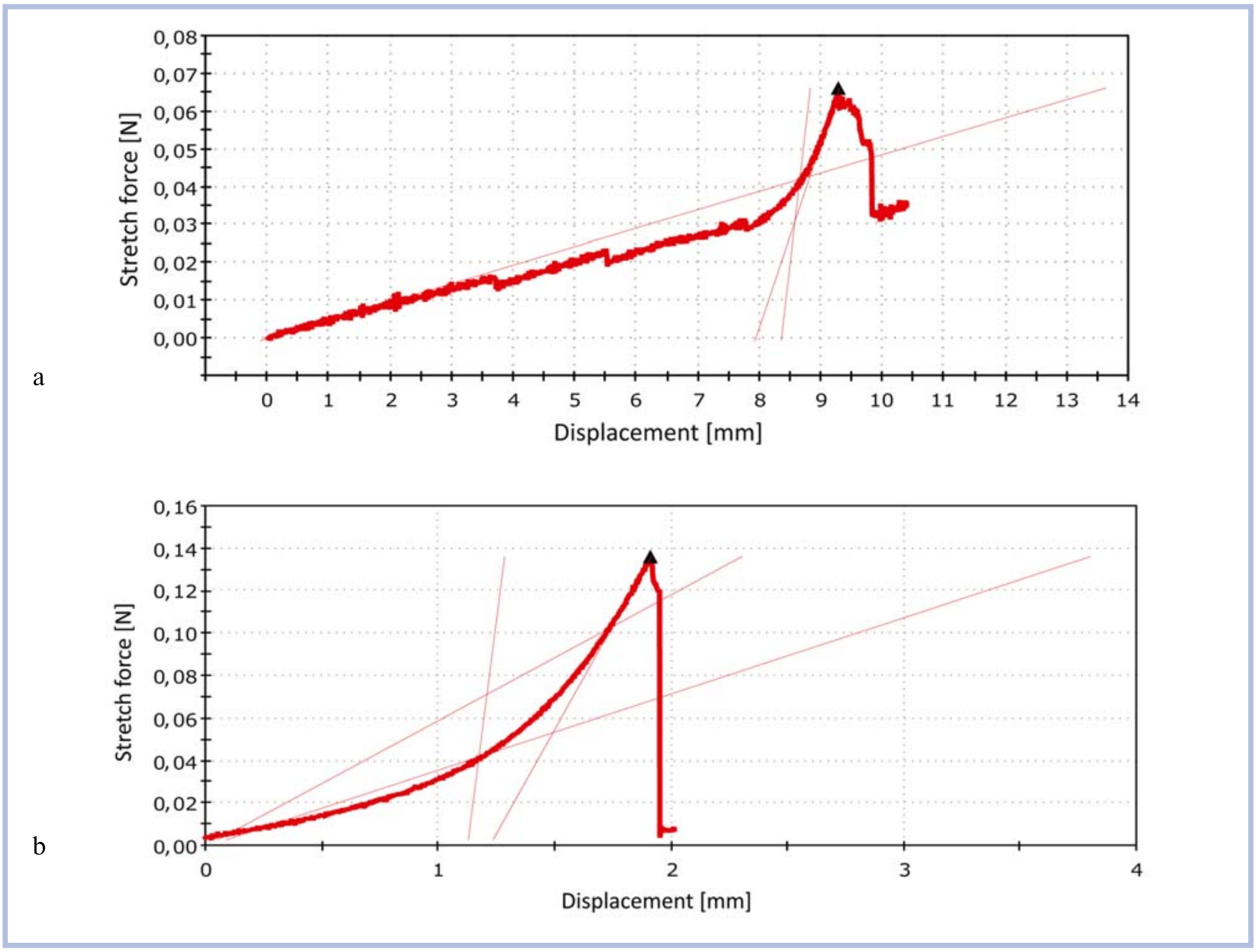

Fig. 3. **Diagrams «stress/stretching displacement» obtained from the mechanical testing of the anterior capsule samples after manual (a) and femtolaser (b) capsulotomy.**

to microgrooves (microtearings) in the spots of impulse laser action and wider deepithelization area compared with the manual technique (**Fig. 4**).

Previous mechanical tests that investigated durability of the peripheral edge of the capsule after manual and femtolaser capsulotomy used porcine eyes as experimental models and their results are rather discrepant. When conducting tests that involve filling the capsular bag with gelatin or hyaluronic acid and then stretching it using retractors, the capsule edge specimens were significantly more durable after laser exposure compared with specimens after manual technique [15, 16]. However, when stretching isolated peripheral ring-shaped specimens of the anterior capsule, the results were opposite: mean strength needed for tearing was significantly higher after manual capsulotomy (155 mN vs 119 mN), and the value was seen to decrease with higher laser energies (from 119 to 108 mN on average) [17, 18]. It is possible that gelatin and hyaluronic acid influenced the mechanical testing process. “Wetting” the capsule edge with such viscous substances may increase its mechanical strength to some extent. The abovementioned microgrooves on the capsule edge found with morphological studies after femtosecond capsulotomy increase the area of capsule edge surface compared with surface area after manual capsulorhexis, which may influence the results of mechanical tests to a greater degree. Besides,

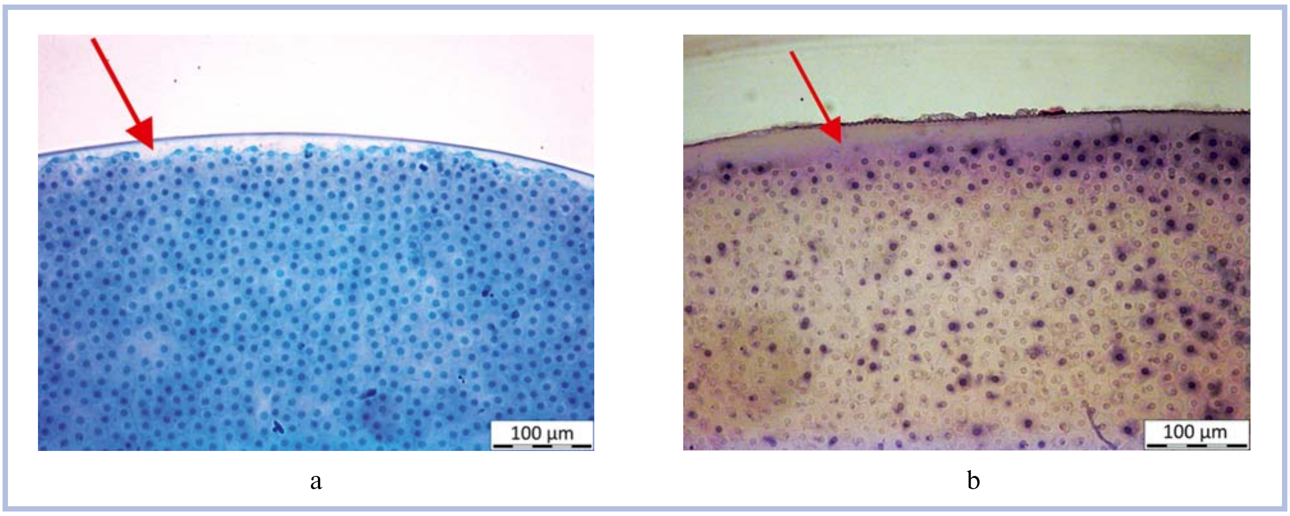

***Fig. 4.* Microsection of the edge of anterior lens capsule after manual (a — Giemsa staining) and femtosecond (b — hematoxylin and eosin staining) capsulotomy.**

Marked with arrows is area of deepithelization. Explanation is given in the text.

when applying research outcomes to clinical practice, one should also consider the differences between porcine and human lens capsule: the former is almost 4 times thicker and much more elastic, its Young's modulus fluctuates between 10.0 and 31.5 N/mm^2 (according to R. Fisher, in human lens the value is 0.7–2.3 N/mm^2). That fact makes the present experimental model inadequate for comparing capsulotomy methods in terms of the clinical application. The influence of these differences on the results of biomechanical studies can be minimised to a certain degree by using young pigs (aged 6–12 months) as experimental animals.

The authenticity of the discrepant results of mechanical tests performed on human cadaver capsule specimens (i.e. obtained *ex vivo*) [19–22] could be influenced by post-mortem changes in the tissues. Only in one research by N. Chan et al. the mechanical testing was done using central portions of the anterior capsule obtained intraoperatively from paired eyes by manual and femtolaser capsulotomy, i.e. in the conditions as close to *in vivo* as possible [24]. The mechanical tests were performed by attaching the specimens between two clamps of the testing device 1.5 mm apart and applying tensile forces. Mean force needed to tear the capsule after manual and laser methods was 2.3 and 2 g, respectively, but the overall difference was not statistically significant. However, it was statistically relevant in 5 cases, which motivated the authors to study those "femtolaser" specimens using scanning electron microscopy – revealing no particular defects of the capsule edges. The provided description of the mechanical test procedure in their study does not elaborate on how the differences in configuration of the "manual" and "laser" specimens were balanced out and, as such, to what extent the results reflect the mechanical properties specifically of the edge portions of the capsule specimens. Obviously, for that reason, when considering the validity of this mechanical test model, the authors provide the following, possibly debatable assumption: "although the *in vivo* capsulotomy is subject to circumferential forces from instruments, this can be seen as a longitudinal stretch when small segments are tested, such as between clamps...".

Conclusions

1. The developed method allows evaluation of mechanical strength of the edge of anterior lens capsule regardless the specimen configuration and in the conditions as close to *in vivo* as possible.

2. Maximum stretching force and extension under maximum stretching force for anterior capsule specimens were significantly higher after manual capsulotomy when compared with ones obtained by femtosecond laser-assisted capsulotomy.

Author contributions:

Study conception and design: K.A., S.A., I.N.

Acquisition and processing of data: K.A., N.B., N.B., I.Kh.

Drafting of manuscript: K.A.

Critical revision: S.A., I.N.

The authors declare that there are no conflicts of interest.

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