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ORIGINAL PAPER

The ultrastructural alterations in the lens capsule and epithelium in eyes with traumatic white cataract

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Abstract
Purpose To demonstrate the morphological and physiological characteristics of lens epithelial cells (LECs) in patients with traumatic cataract using transmission electron microscopy (TEM) to further understand penetrating ocular injury-induced cataract morphology and epithelial repair mechanisms involved at a cellular level.
Methods This is a prospective international study. Sixteen eyes of 16 consecutive patients who were diagnosed as traumatic white cataracts following the anterior lens capsule perforation and 13 eyes of 13 patients with idiopathic posterior subcapsular cataract were included to the study. The anterior lens capsules (aLCs: basement membrane and associated LECs) were obtained from cataract surgery and prepared for TEM.
Results Two prominent cell types were observed in all aLCs of the traumatic cases: degenerated type LECs having variable sized intraepithelial vacuoles close to injury site and normal appearing LECs having an euchromatic nucleus distant from the injury site. In control group, the LECs and all their elements were in normal ultrastructural pattern except some small intraepithelial vacuoles, which were fewer and smaller than the vacuoles in the degenerated LECs of the traumatic group.
Conclusions The ultrastructural findings of our cases support that traumatically induced dysfunction of the lens epithelium may lead to an edema in superficial cortical lens fibers that subsequently undergo degeneration and produce a localized zone of vacuolization.

Keywords Cataract · Electron microscopy · Lens epithelium · Trauma

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Introduction

Ocular trauma includes mechanical eye injury (open and closed globe injury) and non-mechanical eye injury including infrared energy, electric shock, ionizing radiation (X-rays) [1]. Each of these categories can cause traumatic cataract, which might decrease the

vision [2]. Traumatic cataract accounts for 5–10% of all traumatic ocular cases and the incidence of perforating cataract accounts for greater part of the traumatic cataract cases [3].

The pathophysiology of traumatic cataract is complex and has been believed to involve direct rupture of the capsule or coup, countercoup and equatorial expansion due to hydraulic forces transferring the energy of trauma to the opposite side of the eye or the disorders of lens metabolism. The occurrence of traumatic cataract can be observed immediately or several years after the eye injury. Moreover, traumatic cataract may be total or local, in various shapes such as rosette, swollen or other irregular shapes and the location of the opacity may be at anterior or posterior cortex or the capsule [4]. Any of these types of cataract will cause visual problems in the patients.

Although electron microscopy has been used to study the lens materials of human senile cataract, subcapsular cataract, congenital cataract and electric cataract, to the best our knowledge, there has been no electron microscopic study describing the lens capsule and lens epithelium of human traumatic cataract with lens capsule perforation [5–8]. Therefore, the aim of this study was to investigate the ultrastructural characteristics of lens epithelial cells (LECs) in patients with traumatic cataract using transmission electron microscopy (TEM) and thus to better understand the repair mechanisms of lens epithelium at a cellular level in eyes with penetrating ocular injury-induced cataract.

Methods

Study design and patient selection

This prospective international study was performed at the ophthalmology clinic of an Eye Training and Research Hospital from November 2015 to December 2016. The study protocol was approved by the ethics committee. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

The anterior lens capsules (aLCs: basement membrane and associated LECs) were obtained from 16 eyes of 16 consecutive patients who were diagnosed as traumatic white cataracts following the anterior lens capsule perforation (traumatic group—TG) (Fig. 1). The primary repair of the penetrating ocular trauma was performed immediately after the trauma under local anesthesia as an emergency procedure. Cataract surgery and if possible primary intraocular lens implantation were performed as a secondary procedure 24–72 h after the primary surgery. Exclusion criteria included globe rupture, retained intraocular foreign body, a history of blunt trauma without or with lens capsule perforation, uveitis, glaucoma, the anterior segment abnormalities such as keratoconus, Fuchs endothelial dystrophy, fundus abnormalities such as retinal detachment, traumatic macular hole, dense vitreous hemorrhage and systemic associations.

The control group included 13 eyes of 13 patients with idiopathic posterior subcapsular cataract. For the control subjects, patients with any of the following conditions were excluded: a history of any systemic disease and uveitis, ocular trauma or ocular surgery, a history of steroid usage with any form, chronic use of topical ocular medications, patients with an intraocular pressure of > 21 mmHg by Goldmann applanation tonometry: an appearance of glaucomatous optic nerve (cup-to-disk ratio > 0.6, vertical cup asymmetry > 0.2, neuroretinal rim loss or notching), corneal disease, retinal disease and neurological disease or other diseases of the visual pathways.

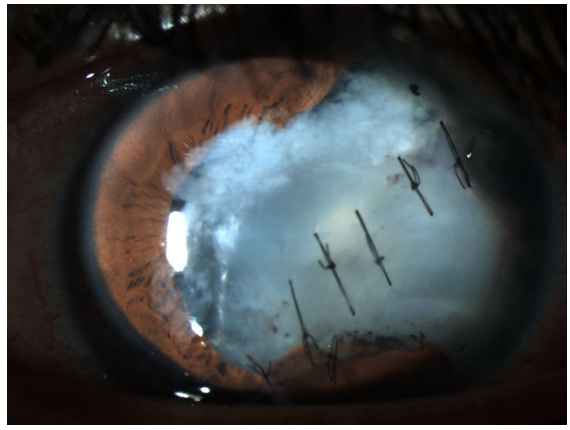

Fig. 1 A slit lamp picture of traumatic white cataract with lens capsule perforation is seen

A detailed history was obtained and a complete ophthalmic examination including the measurement of visual acuity, tonometry, slit lamp biomicroscopy, dilated fundoscopy, B-scan ultrasonography and keratometry, and intraocular lens (IOL) power assessment by SRK-II formula was performed. When it was not possible to obtain the measurements, fellow eye refraction was used to determine the required IOL power.

Surgical technique

The anterior chamber was entered with a 2.8 mm keratome at the limbus. Ophthalmic viscosurgical device was injected into the anterior chamber. All eyes had anterior capsular tear greater than 3 mm. The aLC was grasped from tear region with capsulorhexis forceps, and then, continuous curvilinear capsulorhexis was performed as much as possible. The central circle of the aLC was removed from the eye with the help of a viscoelastic cushion and gentle forceps manipulation to minimize mechanical trauma. The removed aLC materials were sent to the histopathology department immediately after the surgery.

Electron microscopic analysis

The electron microscopic specialist had no information regarding the patient's condition. The tissue samples were put into 2.5% gluteraldehyde for 24 h for primary fixation. Then, these samples were washed with Sorenson's phosphate buffer solution (pH 7.4) and they were post-fixed in 1% osmium tetroxide. After post-fixation, they were washed with the same buffer and dehydrated in increasing concentrations of alcohol series. After dehydration, the tissues were washed with propylene oxide and embedded in epoxy resin embedding media. The semi-thin and ultrathin sections of the obtained tissue blocks were cut with an ultramicrotome (LKB Nova, Sweden). These semi-thin sections, which were two micrometers in thickness, were stained with methylene blue and examined under a light microscope (Nikon, Japan). Following this procedure, trimming was performed on the tissue blocks and their ultrathin sections which were about 60 nm in thickness were taken by the same ultramicrotome. After the staining of these ultrathin sections with uranyl acetate and lead citrate, they were examined under Jeol JEM 1200 EX (Japan) TEM. The electron micrographs of the specimens were taken by the same microscope. The aLC wrinkles were not observed during the preparation of the tissues.

Statistical analysis

Study data were analyzed using the Statistical Package for Social Sciences (SPSS) version 20.0 for Windows (SPSS Inc., Chicago, IL). Descriptive statistics were presented as mean ± standard deviation, frequency distribution and percentages. Pearson Chi-square test and one-sample Chi-square test were used in the analysis of categorical variables. Normal distribution of the variables was tested by visual (histogram and probability graphs) and analytical methods (Kolmogorov–Smirnov/Shapiro–Wilk Test). Independent samples *t* test was used for normally distributed data, and Mann–Whitney U test was used for non-normally distributed data to compare the TG and the control groups. Statistical significance level was set at $p < 0.05$.

Results

This study included 29 eyes of 29 patients; 16 of whom were in the TG and the remaining 13 were in the control group. The TG included 11 men and 5 women with the mean age of 40.62 ± 6.56 years (range, 23–51 years). The control group included 8 men and 5 women with the mean age of 45.07 ± 5.76 years (range, 34–57 years). There were no statistically significant differences regarding the age and sex between the groups ($p > 0.05$). Demographic characteristics of all participants are shown in Table 1.

In the TEM examination of the aLCs, all traumatic cases revealed some significant ultrastructural changes when compared with the control subjects. All eyes had white intumescent cataract in TG, and similar ultrastructural alterations were observed in all aLC samples of TG. It was demonstrated that different cell types were dispersed in the anterior subcapsular region of the capsular perforation area. Two prominent cell types were observed in all aLC samples of the traumatic cases: degenerated type LECs having variable sized intraepithelial vacuoles close to injury site (Fig. 2a, b) and normal appearing LECs having an euchromatic nucleus distant from the injury site

Table 1 Participant characteristics

	Traumatic group ($n = 16$)	Control group ($n = 13$)	p
Age, years (mean ± SD)	40.62 ± 6.56	45.07 ± 5.76	0.066*
Female/male (n/n)	11/5	8/5	0.684**

SD Standard deviation

*Independent samples *t* test

**Chi-square test

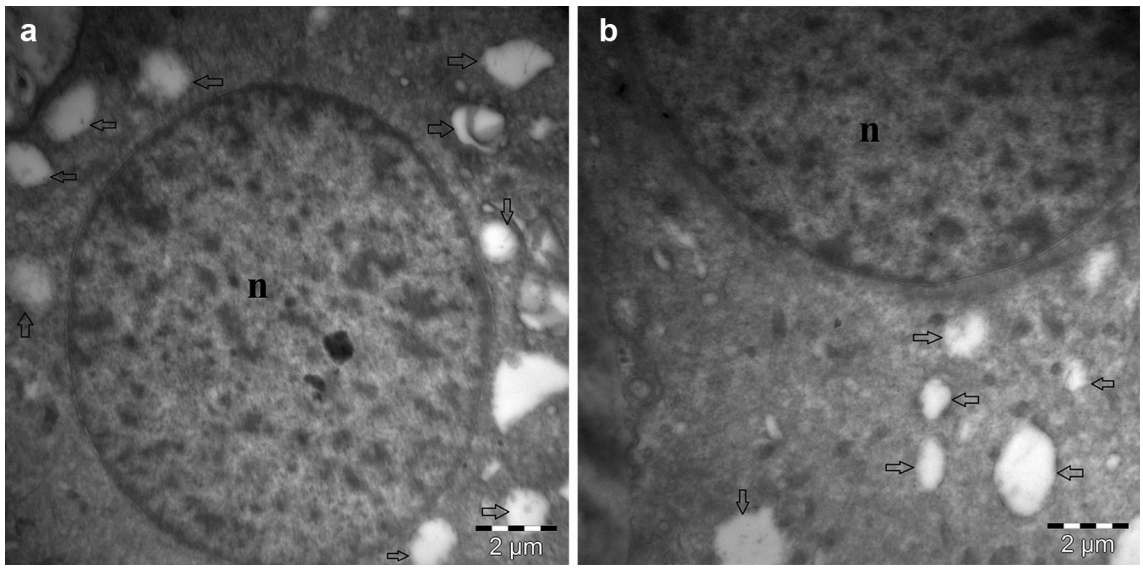

Fig. 2 Electron micrographs of two different patients with white intumescent cataract show some irregular intraepithelial vacuoles and a normal euchromatic nucleus in lens epithelium close to the injury area. The vacuoles are demonstrated with arrows. n, euchromatic nucleus

(Fig. 3a, b). Additionally, single layer of LECs was shown in TG. Conversely, in the control group, the LECs and all their elements were in normal ultrastructural pattern except some small intraepithelial vacuoles (Fig. 4a, b). Also, intraepithelial vacuoles, which were more and larger than the controls, were seen in degenerated LECs of TG.

Discussion

The ultrastructural findings of cataract caused by an ocular perforating injury provide information about the role of the lens epithelium in the self-repair mechanisms of the human lens. The anterior lens capsule and the lens epithelium are the primary site of barrier and active transport and have a crucial role in maintaining the electrolyte and water levels required for lens transparency [9–11]. Disruption of the macromolecular network of the lens capsule and epithelium is predicted to facilitate the passage of water, ions and possibly larger molecules across the basement membrane.

The mechanism of cataract formation caused by an ocular perforating injury in the human lens varies in accordance with the depth and severity of the injury, and there are several forms of cataract, including anterior subcapsular cataract, posterior subcapsular cataract and white cataract. Although various factors, such as the regenerative capacity of LECs and functional recovery of the posterior suture, are known to be significant responses to the swelling of lens

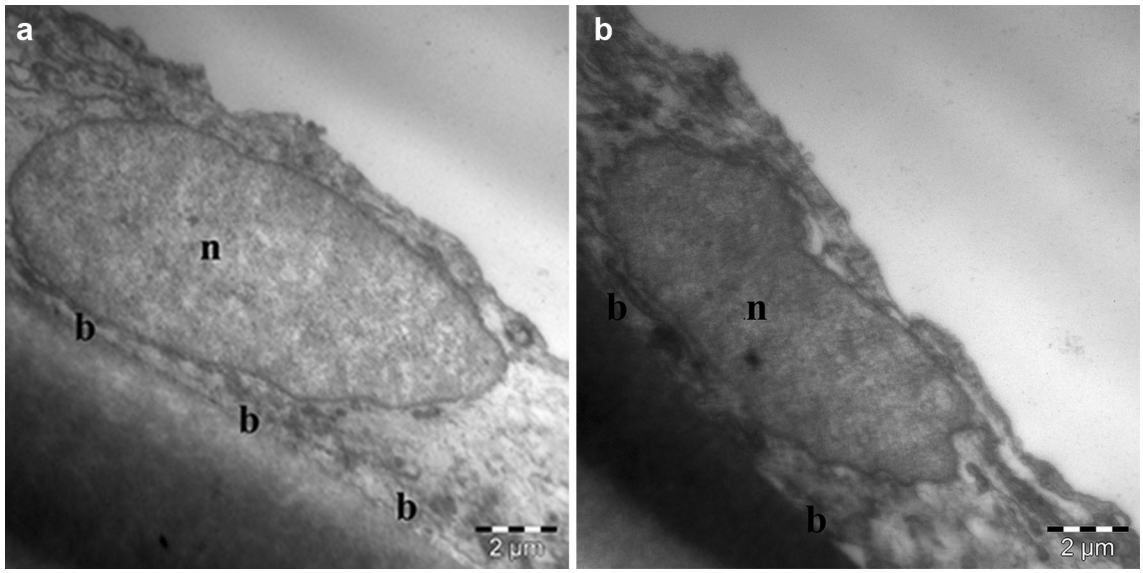

Fig. 3 Electron micrographs of two different patients with white intumescent cataract show normal appearing lens capsule and epithelium with a completely ultrastructural pattern in the specimens taken away from the area of injury. n, nucleus; b, basal lamina

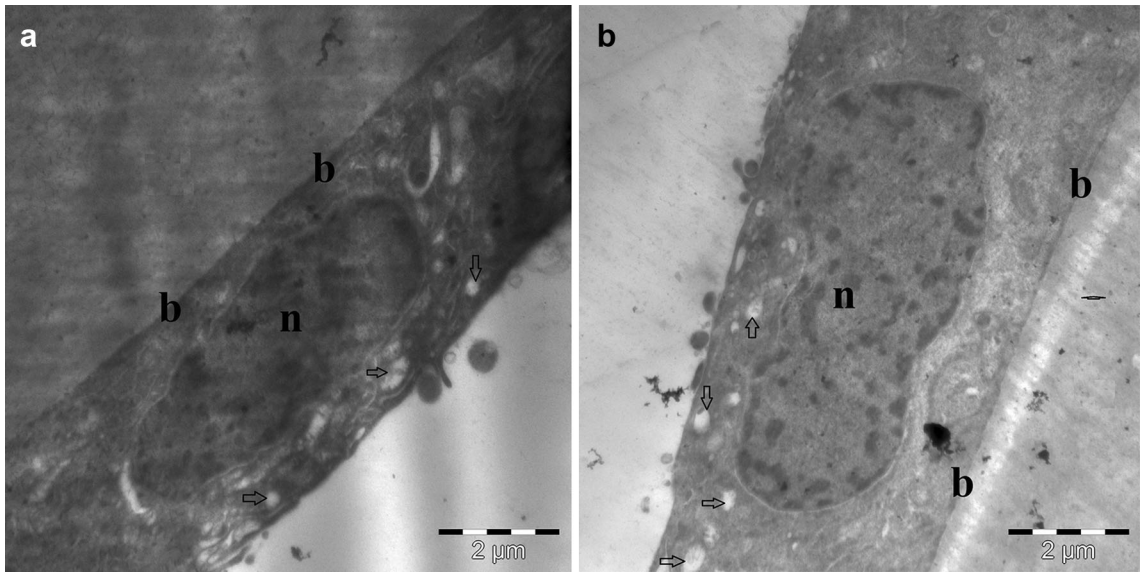

Fig. 4 Electron micrographs of two different patients in the control group revealing some relatively small intraepithelial vacuoles. The lens epithelial cells are normal, flattened, cuboidal and hexagonal in shape, and all of the elements are in a normal ultrastructural pattern, except some small intraepithelial vacuoles. The small vacuoles are demonstrated with arrows. n, nucleus; b, basal lamina

fibers, the precise formative mechanism of traumatic cataract remains unclear [12–15]. Wakasugi et al. [16] investigated the response of the mouse lens to varying sizes of injury and suggested that the injured area of epithelium, the amount of lens fiber damage and the site of liquefaction have a substantial influence on the form and course of cataract formation. The size of the trauma area determined whether the lens recovered or developed opacity, and the severity of epithelial trauma determined the site of lens fiber liquefaction,

which in turn determined the site of onset of opacity. Keeney [17] had contended that penetrating lens injuries might result in cataract if the foreign body caused a capsular tear greater than 3 mm; smaller tears resulted in a localized opacity at the site of injury.

It is thought that cataractogenesis of intumescent white cataract starts as epithelial cell dysfunction firing a cascade of related protein changes in Na–K pump of the LECs [18, 19]. Its dysfunction results in inward osmotic pressure and lens epithelial cells swelling, which does not occur in nuclear cataracts [20]. In our study, all cases had a capsular tear greater than 3 mm resulting in development of a white cataract after 24 h. We thought that a great number of epithelial cells might be damaged with extensive cortical fiber damage and dysfunction of the ion pump mechanisms in the LECs, which have important roles in active uptake of ions and other constituents of the lens, could lead to an influx of aqueous into the lens tissue, subsequently leading to swelling and damage of cortical fiber cells similar to occurring in intumescent cataracts.

In recent years, oxidative damage of the lens has been shown to play an important role in the development of cataract. Studies have shown that oxidative stress and free oxygen radicals can cause a variety of morphological changes in the LECs, including the formation of perinuclear vacuoles and intercellular spaces, and thinning and irregularity of the epithelium in age-related cataracts [21–23]. In our study, intraepithelial vacuoles were observed in both groups; however, degenerative LECs in the TG contained more and larger vacuole structures than the controls. We thought that the intraepithelial vacuoles could be caused by the oxidative stress coming from aging process in both groups and the cell damage due to trauma might cause an increase in the number and the size of vacuoles in degenerated LECs in TG. Observation of the damage only in the trauma zone and not in the trauma free zones may be related to the early cataract surgery.

We also observed single layer of LECs in TG. Gumus et al. [24] reported a case with contusion cataract and they observed that lens epithelium was replaced by multilayered fibrous plaque. We thought that while new layers of basement membrane are formed, degradation of captured LECs may continue through the capsule toward the anterior chamber, those cell stacks will undergo apoptosis and phagocytosis and only a single-layered epithelium will prevail.

In our study, we were also interested to assess whether surgical problems with traumatic white cataract may be associated with ultrastructural morphological changes in anterior capsule and/or lens epithelium. The exact mechanism by which injury stimulus is transmitted to create total epithelial destruction after a local injury is still not known. On the ultrastructural level, human LECs are connected by both gap junctions and desmosomes and contain scattered microfilaments [12]. A change in the tension on the underlying epithelium created by a break in the capsule might conceivably be transmitted from cell to cell along the points of strong attachments, including desmosomes and gap-like junctions. Capsulorhexis may be turn to periphery at such site where these changes are most pronounced, especially if the inner pressure in the lens is high. All of the above may result in higher rate of capsular breaks during the surgery and show the possible connection between morphology and clinical behavior of the aLC in traumatic white cataract. The risk will be minimized with considering these factors during surgery planning. In our study, appropriate precautions were taken during the cataract surgery and no capsular break was observed.

The present study showed that the ultrastructure of the traumatic LECs have remarkable changes such as degenerated LECs with variable sized intraepithelial vacuoles and single layer of LECs. The interest of the present study is not only on a purely histopathologic basis, but also it affords the opportunity to determine the sequence of events in cataractogenesis, since the initial event, the trauma and its exact time are known.

Consequently, the ultrastructural findings of traumatic white cataract can support that dysfunction of the lens epithelium may lead to an edema of superficial cortical lens fibers that subsequently undergo degeneration and produce a localized zone of vacuolization. However, further researches are needed to visualize these dynamic processes in the lens epithelium.

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Compliance with ethical standards

Conflict of interest All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

References

1. Tasman W, Jaegar EA (1997) Traumatic cataract. Duane's. Clin Ophthalmol 1:13–14
2. Khatry SK, Lewis AE, Schein OD, Thapa MD, Pradhan EK, Katz J (2004) The epidemiology of ocular trauma in rural Nepal. Br J Ophthalmol 88:456–460
3. Tsurimaki Y (1993) Traumatic cataract. Current encyclopedia of ophthalmology. Tokyo, Nakayama-Shoten, pp 175–180
4. Shah MA, Shah SM, Shah SB, Patel CG, Patel UA (2011) Morphology of traumatic cataract: does it play a role in final visual outcome? BMJ Open 1:60
5. Meyer LM, Wegener AR, Holz FG, Kronschläger M, Bergmanson JP, Soderberg PG (2014) Ultrastructure of UVR-B-induced cataract and repair visualized with electron microscopy. Acta Ophthalmol 92:635–643
6. Font RL, Brownstein S (1974) A light and electron microscopic study of anterior subcapsular cataracts. Am J Ophthalmol 78:972–984
7. Tekin K, Erol YO, Inanc M, Sargon MF, Can CU, Polat S, Yilmazbas P (2017) Electron microscopic evaluation of anterior lens epithelium in patients with idiopathic congenital cataract. Int Ophthalmol. https://doi.org/10.1007/s10792-017-0713-1 **[Epub ahead of print]**
8. Straatsma BR, Lightfoot DO, Barke RM, Horwitz J (1991) Lens capsule and epithelium in age-related cataract. Am J Ophthalmol 112:283–296
9. Giblin FJ, Chakrapani B, Reddy VN (1976) Glutathione and lens epithelial function. Invest Ophthalmol 155:381–393
10. Bhat SP (2001) The ocular lens epithelium. Biosci Rep 21:537–563
11. Michael R, Bron AJ (2011) The ageing lens and cataract: a model of normal and pathological ageing. Philos Trans R Soc Lond B Biol Sci 366:1278–1292
12. Rafferty NS, Goosens W, March WF (1974) Ultrastructure of human traumatic cataract. Am J Ophthalmol 78:985–995
13. Fagerholm PP, Lundevall E, Trocmé S, Wroblewski R (1986) Human and experimental lens repair and calcification. Exp Eye Res 43:965–972
14. Fagerholm PP, Philipson BT (1979) Experimental traumatic cataract. I. A quantitative microradiographic study. Invest Ophthalmol Vis Sci 18:1151–1159
15. Fagerholm PP, Philipson BT (1979) Experimental traumatic cataract. II. A transmission electron microscopy and extracellular tracer study. Invest Ophthalmol Vis Sci 18: 1160–1171
16. Wakasugi A, Uga S, Sakai Y, Matsumoto M, Hirayama S, Shimizu K (2002) Response of the mouse lens to varying sizes of injured area. Jpn J Ophthalmol 46:391–400
17. Keeney AH (1971) Intralenticular foreign bodies. Arch Ophthalmol 86:499–501
18. Hightower KR (1995) The role of the lens epithelium in development of UV cataract. Curr Eye Res 14:71–78
19. Delamere NA, Tamiya S (2004) Expression, regulation and function of Na, K-ATPase in the lens. Prog Retin Eye Res 23:593–615
20. Brown NP, Bron AJ (1996) Lens disorders: a clinical manual of cataract diagnosis. Butterworth-Heinenmann, Oxford, pp 11–80
21. Cheng HM, Fagerholm P, Chylack LT Jr (1983) Response of the lens to oxidative-osmotic stress. Exp Eye Res 37:11–21
22. Nita M, Grzybowski A (2016) the role of the reactive oxygen species and oxidative stress in the pathomechanism of the age-related ocular diseases and other pathologies of the anterior and posterior eye segments in adults. Oxid Med Cell Longev 2016:3164734
23. Berthoud VM, Beyer EC (2009) Oxidative stress, lens gap junctions, and cataracts. Antioxid Redox Signal 11:339–353
24. Gumus K, Kiratli H, Yuruker S (2006) Ultrastructural study of a contusion cataract. Indian J Ophthalmol 54:215–216