Human lens epithelium structural and functional studies in association with cataract formation.

Sofija Andjelic*, Marko Hawlina

Department of Ophthalmology, Eye Hospital, University Medical Centre, Ljubljana, Slovenia

Received: *10-Feb-2023, Manuscript No. AACOVS-23-89465;* ***Editor assigned:*** *13-Feb-2023, PreQC No. AACOVS-23-89465 (PQ);* ***Reviewed:*** *27-Feb-2023, QC No. AACOVS-23-89465;* ***Revised:*** *06-Mar-2023, Manuscript No. AACOVS-23-89465 (R);* ***Published:*** *13-Mar-2023, DOI: 10.35841/aacovs.7.5.411-415*

Abstract

This review summarizes recent work related to the structure and the function of the human anterior lens epithelium and its role in the formation of cataract, one of the central issues in eye research. It emphasizes that the human lens capsule preparation isolated during the cataract surgery is an adequate source for the studies of lens epithelial cells and it highlights the possibilities for studying the changes in intracellular calcium homeostasis, macromolecular components and structure of lens epithelium associated with cataract.

Keywords: Lens epithelial cells, Lens epithelium, Structure, Function, Cataract, Posterior capsule opacification, Translational research.

Introduction

Cataract, the opacity of the crystalline lens, is the main cause of avoidable blindness being responsible for 48% of world blindness [1]. Since 2000, an increase of 30% in cases of cataract blindness and 93% cases of moderate and severe vision impairment has been reported [2]. In 2020, approximately 15 million people aged over 50 years were blind worldwide, with further 80 million having moderate and severe vision impairment due to cataract. In addition, posterior capsule opacification (PCO) often named "secondary cataract," is the most frequent complication after cataract surgery, developing in approximately 20% of cases within a period of five years [3].

Literature Review

Cataracts can be classified by the age at onset: a congenital or infantile cataract presents within the first year of life; a juvenile cataract presents within the first decade of life; a presenile cataract presents before the age of about 45 years, and age-related or senile cataract after that [4]. The secondary cataract can be associated with ocular conditions such as retinitis pigmentosa or uveitis, or systemic conditions as in the case of diabetes, or can be also drug-induced, mainly by steroids [5,6].

Understanding the biology of cataractogenesis, the process of cataract formation is, therefore, a high-priority goal. Based on the part where the lens opacification develops, cataracts are principally divided into three types: nuclear (N), opacification in the lens' center; cortical (C), opacification in the lens' outer layers and posterior subcapsular cataracts (PSC), while the combined N+C cataracts occur frequently [7].

The lens has three main parts: the capsule, the epithelium, and the fibers. The lens capsule or basal lamina is the outer lens' layer that surrounds the lens. The firmly packed lens fibre cells make the main body of the lens' interior. The single-layered lens epithelium, made by lens epithelial cells (LECs), is situated in the anterior part of the lens between the capsule and the fibers [8,9].

The lens epithelium is a lens' physical and biological boundary and is the lens' metabolically most active region that regulates nearly all of the lens' homeostatic functions. LECs are very important for lens transparency preservation because of their roles in whole-lens energy production, antioxidative mechanisms and biochemical transport [10, 11]. Also, PCO usually develops from the LECs that remain in the lens capsule after cataract surgery. LECs proliferation, migration and transformation (i.e., epithelial–mesenchymal transition (EMT)) are critical elements in PCO development [12-14].

Continuous research in different laboratories are focused on understanding lens epithelium functioning in the context of cataractogenesis and PCO, their prevention and treatment. In recent years one of the biggest progress in lens research was done by showing that the functional lens can regenerate only from endogenous LECs [15] but this principle has not as yet been widely clinically applied. The studies concerning the mechanisms of response to oxidative stress acting on lens epithelium are also in a focus of lens research with an extensive number of publications, some of which are in connection with age-related cataract attenuation [16,17], some with the EMT [18-21] and its attenuation [22-24]. The radiation effect on LECs is also studied [25-27]. Recent studies also focused on the role of connexin [28,29], calcium [30], Transient receptor potential vanilloid (TRPV) channels [31] and autophagy [32,33] related to lens epithelium. The lens epithelia structural changes associated with pathology have also been studied [34]. Some of the recent reviews relate to the role of ion transport regulation and TRPV (Transient receptor potential vanilloid) channels [35], oxidative stress and connexin [36], lens biochemistry [37], autophagy [38,39], ionizing radiation [40,41], EMT and cell adhesion signaling [42] and PCO [43].

Recent structural and functional studies of the lens epithelia increase the knowledge about the lens epithelia in different cataract pathological states helping in understanding the role of

lens epithelia in cataractogenesis, PCO and lens regeneration.

Multiple complementary techniques for studying the lens epithelium

The innovative integration of multiple complementary state-of-the-art approaches is necessary to address the role of the lens epithelium in cataract. Attempts to understand aspect of the human lens epithelium were done also in our laboratory by conducting functional studies by calcium (Ca^{2+}) imaging [44-47], structural studies by scanning electron microscopy (SEM), transmission electron microscopy (TEM) and confocal microscopy [9,48-50] and FTIR micro-spectroscopic studies [51,52]. The primary LECs cultures [53-55] were also studied for understanding the PCO and lens regeneration.

Human anterior lens capsule preparation consisting of the monolayer of anterior LECs lying on the basal lamina were used. Cataractous human anterior lens capsules are excised during routine cataract surgeries by the capsulorhexis technique and are also classified based on the level of cataract development by a modified LOCS III grading system from 1 to 5, where 1 reflects the minimal level of cataract and 5 is the highest [56]. Non-cataractous, control, healthy human anterior lens capsules derive from patients having primary pathology in the posterior eye' segment. The central anterior lens capsules' circles 5-5.5 mm in diameter are extracted by continuous curvilinear capsulorhexis. The lens capsules are dissected in a way that their anterior portion (i.e., basal lamina and attached LECs) are separated from the fiber cells that form the lens' main body. The lens capsules would normally be discarded but instead, they represent a potential source of the human lens epithelium that offers the possibility to study the physiology and pathophysiology of human LECs. The method is explained in detail in Andjelic et al. [57]. Working on the human preparation obtained immediately after the surgery allows direct studies of the tissue of interest in different forms of cataracts and the obtained results are directly applicable to increase our knowledge about the cataract pathophysiology.

Discussion

The structural and functional characteristics of the human lens epithelium were studied in normal, healthy epithelia as well as in different cataract' types.

Structural studies: The human lens epithelium' and LECs' structure together with the differences between the apical and basal sides were shown by using scanning electron microscopy (SEM), transmission electron microscopy (TEM), and confocal microscopy. The specific morphological characteristics were shown using these three methods: the LECs' cytoplasmic membrane's extensions and the entanglements at the boundary with the lens capsule, while the LECs' apical surface of LECs was shown to be smooth [9]. Using SEM and TEM, the lens epithelium was studied in patients with diverse cataract types and in cataracts connected with diverse diseases. In presenile cataract, the most important anomalous characteristics discovered by SEM were the changes in the LECs structure with the dents and the selective concavity of some LECs at their apical side centrally toward the nucleus. Additionally, TEM displayed the thinning of the lens epithelium with the segmentally concave cells and the compressed and elongated nuclei [48]. In patients with intumescent cataracts, the most noticeable anomalous lens epithelium characteristics were swollen cells, spherical formations and degraded cells [50,58]. In patients with uveitis, predominately in white uveitic cataracts, extensive epithelial and capsular-epithelial border changes and EMT in some fibrotic capsules were found [59]. In patients with retinitis pigmentosa, the anomalous characteristics discovered were mainly holes, thinning and degradation of the epithelium, with size from <1 μm to more than 50 μm. Other types of holes in dimensions up to 20 μm were observed that may be created by the gradual stretching of the lens epithelium. Other types of anomalous characteristics were cracks that were visible between neighboring LECs, with sizes of 0.1–2 μm × up to 10 μm [49]. The holes in the lens epithelium may have a role in cataract development.

A method for forming adherent ex-vivo lens explant cultures was developed [55]. By using SEM, the distribution of the cultured LECs and their morphology including gradual formation of a lateral connection between the LECs were shown. Utilizing cultured human anterior lens capsule explants and visualizations by light microscopy, SEM and immunofluorescence staining for proliferation and pluripotency markers, it was demonstrated that the human anterior lens capsule has LECs that can proliferate and migrate, indicating that not only equatorial LECs can do so, but also anterior LECs [53]. It was also shown that a focused and highly-localized atmospheric pressure microplasma jet with electrode discharge could induce dose-dependent apoptosis in chosen and targeted individual LECs, which is of importance as a potential treatment against PCO development [54].

Functional studies: The role of Ca^{2+} in cataract formation has also been studied. Homeostasis of the intracellular Ca^{2+} concentration [Ca^{2+}] is a general indicator of the functioning of cells. For investigating the cellular Ca^{2+} dynamics, the most often applied technique is the use of Ca^{2+} indicator dyes. They allow the following of spatio-temporal changes in [Ca^{2+}] in real-time. Fura-2 AM, a cell-permeable dye, is used in order to study the role of the altered intra as well as inter-cellular Ca^{2+} signaling in LECs and the subsequent effect this may have in cataractogenesis.

We studied how Ca^{2+} homeostasis modifications in human anterior LECs are connected with diverse cataract types (C or N) and how the cataract progression (mild or moderate) influences the Ca^{2+} signaling. The intra and intercellular Ca^{2+} signaling in human anterior lens capsules' LECs after the application of agonist acetylcholine (ACh) was investigated, and it was demonstrated that in more developed cataracts, cells manifest a slower collective response to stimulation and a less pronounced spatiotemporal clustering of LECs. The intercellular networks were also sparser and more segregated than in mild cataracts. In addition, it was demonstrated that spontaneously active LECs often operate in localized groups with quite well-aligned Ca^{2+} activity. The presence of spontaneous activity was also discovered to influence the stimulated Ca^{2+} responses of individual LECs. These findings point out that the cataract progression involves intercellular signaling' impairment therewith suggesting the functional importance of changed Ca^{2+} signaling of LECs in cataractogenesis [45].

The spatiotemporal changes in Ca^{2+} signaling in LECs upon local mechanical stimulation were also investigated, to better understand the LECs' intercellular communication and its association with cataractogenesis. It was shown that the Ca^{2+} signal spreads radially from the stimulation point and that the amplitude of Ca^{2+} transients decreases with increasing distance. The comparison of signaling characteristics with respect to the cataract progression level showed that in lens epithelia from more developed cataracts, the Ca^{2+} wave propagates faster and the amplitudes of Ca^{2+} signals are lower, while their durations are longer. However, when comparing lens epithelia with regard to the cataract type, no differences were found. Additionally, experiments with antagonist apyrase showed that the Ca^{2+} signals are not influenced by ATP-dependent paracrine communication. The results again indicated that cataract progression is connected with changes in Ca^{2+} signaling in LECs, suggesting the functional importance of changed Ca^{2+} signaling of LECs in cataractogenesis [44].

The contraction of LECs upon nonspecific stimulation, which is at least partly independent of the changes in intracellular Ca^{2+} concentration was also shown. Contraction can be induced by a mechanical stimulus, where the response is fast and after the termination of stimulation, cells tend to return to the initial noncontracted state [46]. This contraction of LECs can lead to higher water permeability, which could be the mechanism of the formation of cataracts upon the insertion of the phakic lens if the latter are touching the anterior lens capsule.

Macromolecular studies: The macromolecular cell components in connection with cataractogenesis were also studied. For this scope, the synchrotron radiation-based Fourier Transform Infrared (SR-FTIR) micro-spectroscopy, a state-of-the-art vibrational spectroscopic technique that is a powerful tool for cell components analysis, e.g. lipids, proteins and nucleic acids, was used. The SR-FTIR micro-spectroscopy setup installed on the beamline MIRAS eventualy "Infrared Microspectroscopy beamline at the Spanish synchrotron light source ALBA in Barcelona was used, where the measurements were set to reach a single-cell resolution. The spectroscopic advantage lies in the fact that the chemical change precedes or accompanies any morphological change that is symptomatic of cataracts. When comparing the N and C cataract lens epithelia by using SR-FTIR, it was shown that protein aggregation in form of fibrils was strong in LECs of N cataracts, while oxidative stress and lipids peroxidation were more noticeable in LECs of C cataracts [52]. UV-C irradiation of lens capsules had a significant effect on protein conformation with protein formation of intramolecular parallel β-sheet structure, lower phosphate and carboxyl bands in fatty acids and amino acids, and oxidative stress markers with a significant rise of lipid peroxidation and diminish of the asymmetric CH3 band [51-61].

Conclusions

There are many advantages in using human intraoperatively removed lens capsule and the primary culture of LECs for the studies of mechanisms involved in cataract, PCO and lens regeneration. Firstly, the lens capsules with the monolayer of LECs are regularly excised and normally discarded during cataract surgery, so there is a steady supply of human lens capsule material. Immediate use of this material allows studies of the physiology of living LECs with Ca^{2+} imaging. FTIR and structural studies do not require live tissue, which allows the studies to be done at a remote location with state-of-the-art equipment. Secondly, the lens capsule preparation has the advantage of preserving the epithelium in a fairly "intact" configuration, i.e. all, or at least most, of the connections between neighboring LECs and to the underlying basement membrane are preserved and if the preparation was not mishandled they should behave as they do in their normal environment. This is not the case with the intercellular connections of cultured LECs. However, primary LECs cultures are a model for lens regeneration and PCO formation, its prevention and treatment, where it can be studied how the LECs start to migrate, reconnect and resemble native lens epithelial tissue. There is also the difference in receptor subtype expression found between native and cultured LECs, for example in the human lens epithelial cells line HLE -B3, it is the muscarinic receptorM3 subtype that predominates and not M1 as in the native epithelium. Regarding animal models, no single animal species is a complete model of the human lens. There can be differences in receptor subtype expression even when the same agonist induces responses in different species. For example, in the case of muscarinic receptors, the native human lens cells express the M1 subtype, whereas rats and rabbits express the M3 as the dominant subtype. As the experimental conclusions obtained on animal species cannot be directly applied to humans, working on the human preparation obtained during the surgery, allows direct studies of the tissue of interest in different forms of cataracts and the results obtained are directly applicable to enhance our understanding of the cataract pathophysiology, its prevention and treatment.

References

1. Klein BE, Klein R, Lee KE. Incidence of age-related cataract over a 10-year interval:the Beaver Dam Eye Study. Ophthalmology. 2002;109:2052-2057.
2. Pesudovs K, Lansingh VC, Kempen JH, et al. Cataract-related blindness and vision impairment in 2020 and trends over time in relation to VISION 2020:the Right to Sight:an analysis for the Global Burden of Disease Study. ARVO. 2021;62:8.
3. Wormstone IM. Posterior capsule opacification:a cell biological perspective. Exp Eye Res. 2002;74:337-347.
4. Hejtmancik JF. Congenital cataracts and their molecular genetics. Semin Cell Dev Biol. 2008;19:134-149.
5. Gupta VB, Rajagopala M, Ravishankar B. Etiopathogenesis of cataract:an appraisal. Indian J Ophthalmol. 2014;62:103-110.
6. Andjelić S, Hawlina M. Cataractogenesis. Zdrav Vestn-Slovenian Medical J. 2012;81:I122-I132.
7. Gupta PD, Johar K, Vasavada A. Causative and preventive action of calcium in cataracto-genesis. Acta Pharmacol Sin. 2004;25:1250-1256.
8. Hejtmancik JF, Shiels A. Overview of the Lens. Prog Mol Biol Transl Sci. 2015;134:119-127.
9. Andjelic S, Drašlar K, Hvala A, et al. Anterior lens epithelial cells attachment to the basal lamina. Acta Ophthalmol. 2016;94:e183-8.

10. Bhat SP. The ocular lens epithelium. Biosci Rep. 2001;21:5375-5463.
11. Mathias RT, Rae JL. The lens:local transport and global transparency. Exp Eye Res. 2004;78:689-698.
12. Raj SM, Vasavada AR, Johar SR, et al. Post-operative capsular opacification:a review. Int J Biomed Sci. 2007;3:237-250.
13. Wormstone IM, Wang L, Liu CSC. Posterior capsule opacification. Exp Eye Res. 2009;88:257-269.
14. Wormstone IM, Eldred JA. Experimental models for posterior capsule opacification research. Exp Eye Res. 2016;142:2-12.
15. Lin H, Ouyang H, Zhu J, et al. Lens regeneration using endogenous stem cells with gain of visual function. Nature. 2016;531:323-328.
16. Ran H, Liu H, Wu P. Echinatin mitigates H2O2-induced oxidative damage and apoptosis in lens epithelial cells via the Nrf2/HO-1 pathway. Adv Clin Exp Med. 2021;30:1195-1203.
17. Fan Q, Li D, Zhao Z, et al. Protective effect of Glutaredoxin 1 against oxidative stress in lens epithelial cells of age-related nuclear cataracts. Mol Vis. 2022;28:70-82.
18. Chen X, Yan H, Chen Y, et al. Moderate oxidative stress promotes epithelial–mesenchymal transition in the lens epithelial cells via the TGF-β/Smad and Wnt/β-catenin pathways. Mol Cell Biochem. 2021;476:1631-1642.
19. Wang R, Li J, Zhang X, et al. Extracellular vesicles promote epithelial-to-mesenchymal transition of lens epithelial cells under oxidative stress. Exp Cell Res. 2021;398:112362.
20. Wei Z, Gordon P, Hao C, et al. Aged Lens Epithelial Cells Suppress Proliferation and Epithelial-Mesenchymal Transition-Relevance for Posterior Capsule Opacification. Cells. 2022;11:2001.
21. Li, X, Sun, M, Cheng, et al. LncRNA GAS5 regulates migration and epithelial-to-mesenchymal transition in lens epithelial cells via the miR-204-3p/TGFBR1 axis. Lab Invest. 2022;102:452-460.
22. Sugiyama Y, Nakazawa Y, Sakagami T, et al. Capsaicin attenuates TGFβ2-induced epithelial-mesenchymal-transition in lens epithelial cells in vivo and in vitro. Exp Eye Res. 2021;213:108840.
23. Fu J, Hu X. Simvastatin alleviates epithelial-mesenchymal transition and oxidative stress of high glucose-induced lens epithelial cells in vitro by inhibiting RhoA/ROCK signaling. Exp Ther Med. 2022;23:420.
24. Huang J, Chen Z, Lai Z, et al. Kaempferol ameliorates the regulatory effects of PVT1/miR-214 on epithelial-mesenchymal transition through the PAK4/β-catenin axis in SRA01/04 cells. Future Med Chem. 2021;13:613-623.
25. Barnard SGR, McCarron R, Mancuso M, et al. Radiation-induced DNA Damage and Repair in Lens Epithelial Cells of both Ptch1(+/-) and Ercc2(+/-) Mutated Mice.V Radiat Res. 2022;197:36-42.
26. Vigneux G, Pirkkanen J, Laframboise T, et al. Radiation-Induced Alterations in Proliferation, Migration, and Adhesion in Lens Epithelial Cells and Implications for Cataract Development. Bioengineering (Basel). 2022;9:29.
27. Wu Q, Song J, Gao Y, et al. Epigallocatechin gallate enhances human lens epithelial cell survival after UVB irradiation via the mitochondrial signaling pathway. Mol Med Rep. 2022;25:87.
28. Quan Y, Du Y, Wu C, et al. Connexin hemichannels regulate redox potential via metabolite exchange and protect lens against cellular oxidative damage. Redox Biol. 2021;46:102102.
29. Tjahjono N, Xia CH, Li R, et al. Connexin 50-R205G Mutation Perturbs Lens Epithelial Cell Proliferation and Differentiation. Invest Ophthalmol Vis Sci. 2020;61:25.
30. Gao C, Liu X, Fan F, et al. Exosomal miR-29b found in aqueous humour mediates calcium signaling in diabetic patients with cataract. Int J Ophthalmol. 2021;14:1484-1491.
31. Chen L, Chen Y, Ding W, et al. Oxidative Stress-Induced TRPV2 Expression Increase Is Involved in Diabetic Cataracts and Apoptosis of Lens Epithelial Cells in a High-Glucose Environment. Cells. 2022;11:1196.
32. Khan SY, Ali M, Kabir F, et al. The role of FYCO1-dependent autophagy in lens fiber cell differentiation. Autophagy. 2022:1-18.
33. Huang J, Yu W, He Q, et al. Autophagy facilitates age-related cell apoptosis-a new insight from senile cataract. Cell Death Dis. 2022;13:37.
34. Sorkou KN, Manthou ME, Meditskou S, et al. Lens Epithelial Surface Disorders in Exfoliation Syndrome:A Scanning and Transmission Electron Microscopy Study. Ophthalmic Res. 2021;64:216-223.
35. Delamere NA, Shahidullah M. Ion Transport Regulation by TRPV4 and TRPV1 in Lens and Ciliary Epithelium. Front Physiol. 2022;12:834916.
36. Quan Y, Du Y, Tong Y, et al. Connexin Gap Junctions and Hemichannels in Modulating Lens Redox Homeostasis and Oxidative Stress in Cataractogenesis. Antioxidants (Basel). 2021;10:1374.
37. Muranov KO, Ostrovsky MA. Biochemistry of Eye Lens in the Norm and in Cataractogenesis. Biochemistry (Mosc). 2022;87:106-120.
38. Fernández AJA, de Julián LE, Soler DC, et al. The Role of Autophagy in Eye Diseases. Life (Basel). 2021;11:189.
39. Brennan L, Disatham J, Kantorow M. Mechanisms of organelle elimination for lens development and differentiation. Exp Eye Res. 2021;209:108682.
40. Barnard SGR, Hamada N. Individual response of the ocular lens to ionizing radiation. Int J Radiat Biol. 2022:1-17.
41. Ainsbury EA, Barnard SGR. Sensitivity and latency of ionising radiation-induced cataract. Exp Eye Res. 2021;212:108772.
42. Taiyab A, West-Mays J. Lens Fibrosis:Understanding the Dynamics of Cell Adhesion Signaling in Lens Epithelial-Mesenchymal Transition. Front Cell Dev Biol. 2022;10:886053.
43. Wormstone IM, Wormstone YM, Smith AJO, et al. Posterior capsule opacification:What's in the bag? Prog Retin Eye Res. 2021;82:100905.
44. Gosak M, Gojić D, Spasovska E, et al. Cataract Progression Associated with Modifications in Calcium Signaling in Human Lens Epithelia as Studied by Mechanical Stimulation. Life (Basel). 2021;11:369. [

45. Gosak M, Markovič R, Fajmut A, et al. The Analysis of Intracellular and Intercellular Calcium Signaling in Human Anterior Lens Capsule Epithelial Cells with Regard to Different Types and Stages of the Cataract. PLoS One. 2015;10:e0143781.
46. Andjelic S, Zupančič G, Perovšek D, et al. Human anterior lens capsule epithelial cells contraction. Acta Ophthalmol. 2011;89:e645-53.
47. Andjelic S, Zupančič G, Perovšek D, et al. Anterior lens capsule as a tool to study the physiology of human lens epithelial cells. Zdrav Vestn-Sloven Med J. 2010;79:123-130.
48. Andjelic S, Drašlar K, Hvala A, et al. Structural Characteristics of the Lens in Presenile Cataract. Front Med (Lausanne). 2021;8:802275.
49. Andjelic S, Drašlar K, Hvala A, et al. Anterior lens epithelium in cataract patients with retinitis pigmentosa - scanning and transmission electron microscopy study. Acta Ophthalmol. 2017;95:e212-e220.
50. Andjelic S, Drašlar K, Hvala A, et al. Anterior lens epithelium in intumescent white cataracts - scanning and transmission electron microscopy study. Graefes Arch Clin Exp Ophthalmol. 2016;254:269-276.
51. Lumi X, Dučić T, Kreuzer M, et al. UV Effect on Human Anterior Lens Capsule Macro-Molecular Composition Studied by Synchrotron-Based FTIR Micro-Spectroscopy. Int J Mol Sci. 2021;22:5249.
52. Kreuzer M, Dučić T, Hawlina M, et al. Synchrotron-based FTIR microspectroscopy of protein aggregation and lipids peroxidation changes in human cataractous lens epithelial cells. Sci Rep. 2020;10:15489.
53. Andjelić S, Drašlar K, Lumi X, et al. Morphological and proliferative studies on ex vivo cultured human anterior lens epithelial cells - relevance to capsular opacification. Acta Ophthalmol 2015;93:e499-506.
54. Recek N, Andjelic S, Hojnik N, et al. Microplasma Induced Cell Morphological Changes and Apoptosis of Ex Vivo Cultured Human Anterior Lens Epithelial Cells - Relevance to Capsular Opacification. PLoS One. 2016;11:e0165883.
55. Andjelic S, Lumi X, Veréb Z, et al. A simple method for establishing adherent ex vivo explant cultures from human eye pathologies for use in subsequent calcium imaging and inflammatory studies. J Immunol Res. 2014;2014:232659.
56. Chylack LT, Wolfe JK, Singer DM, et al. The lens opacities classification system III. The longitudinal study of cataract study group. Arch Ophthalmol. 1993;111:831–836.
57. Andjelic S, Zupančič G, Hawlina M. The preparations used to study calcium in lens epithelial cells and its role in cataract formation. J Clin Exp Ophthalmol. S1:002.
58. Hawlina M, Stunf S, Hvala A. Ultrastructure of anterior lens capsule of intumescent white cataract. Acta Ophthalmol. 2011;89:e367-70.
59. Stunf S, Hvala A, Vidovič VN, et al. Ultrastructure of the anterior lens capsule and epithelium in cataracts associated with uveitis. Ophthalmic Res. 2012;48:12-21.
60. Collison DJ, Coleman RA, James RS, et al. Characterization of muscarinic receptors in human lens cells by pharmacologic and molecular techniques. Invest Ophthalmol Vis Sci. 2000;41:2633-2641.
61. Rhodes JD, Thomas G, Duncan G. Acetylcholine-induced electrical responses in intact human, rat and rabbit lenses. Exp Eye Res. 2002;74:417-421.

*Correspondence to:

Dr. Sofija Andjelic

Department of Ophthalmology,

Eye Hospital, University Medical Centre,

Ljubljana, Slovenia.

E-mail: sofija.andjelic@kclj.si

Tel: +386 1 522 19 29