The role of lens epithelial cells in the development of the posterior capsule opacification and in the lens regeneration after congenital cataract surgery

Sofija Andjelic

Department of Ophthalmology, University Medical Centre Ljubljana, Ljubljana

Correspondence:
Sofija Andjelic,
e: sofija.andjelic@kclj.si

Key words:
lens epithelial cells; lens capsular bag; cell pluripotency; cell proliferation; migration of cells

Cite as:
Zdrav Vestn. 2017; 86:236–43.

Received: 16. 11. 2016
Accepted: 6. 4. 2017

Abstract

Posterior capsule opacification–PCO is the most common complication after cataract surgery. Proliferation and migration of lens epithelial cells that remain in the capsular bag following cataract surgery can lead to the development of PCO, which is the main cause of deterioration of visual function. PCO shows the classic features of fibrosis, including hyperproliferation, migration, deposition of matrix and its shrinkage and transdifferentiation into myofibroblast.

Astonishingly, the results of recent research show the importance of lens epithelium for lens regeneration following congenital cataract surgery. New minimally invasive cataract surgery removes only 1–1.5 mm of lens epithelium more laterally, so the major part of the epithelium remains in the capsular bag. Conceptually, the new method differs from the current practice, since it preserves the endogenous epithelial cells of the lens and achieves functional lens regeneration in rabbits and monkeys as well as in human infants with congenital cataracts. Pluripotency of lens epithelial cells and their stem cell nature are crucial for lens regeneration.

Ex vivo cultures of the lens capsule explants can be used for testing the pharmacological agents for stimulating and inhibiting the growth of lens epithelial cells. Functionality of the cells and responses to pharmacological agents can be studied by analysing the intra- and inter-cellular calcium (Ca^{2+}) signalling. Stimulating the growth of lens epithelial cells is important in lens regeneration while inhibiting the growth of lens epithelial cells is important in preventing the development of PCO. In the article I described the methods for the analysis of lens epithelial cells after cataract surgeries, which are carried out in the laboratory of the Eye Hospital, University Medical Centre Ljubljana.

Cite as: Zdrav Vestn. 2017; 86:236–43.

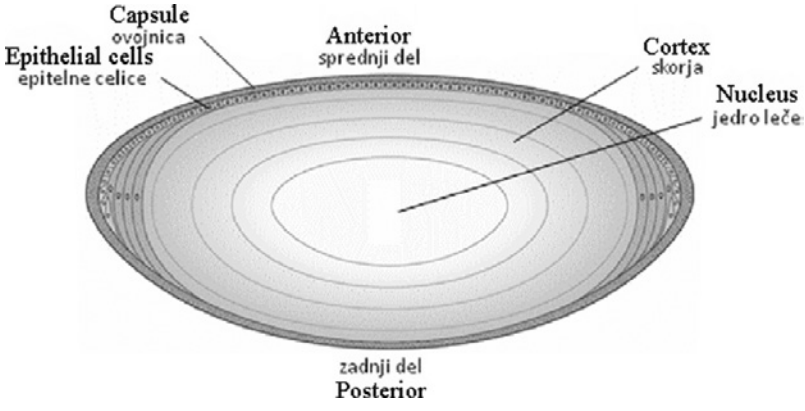
1. Introduction

Cataracts are the most common cause of vision impairment in humans. One talks about cataract, when the transparency of the lens is reduced, which prevents the light from smooth transition to the back of the eye, thereby worsening vision. According to the World Health Organization, it is estimated that about 20 million people today have bilateral visual impairment due to cataract, this number is expected to reach 50 million by 2050 (1). Currently, the main method of treatment is surgical, so the cataract surgery is the most common eye surgery.

The lens is composed of three parts: the capsule, the cortex and the nucleus (Figure 1). The lens capsule is a thin transparent membrane that wraps the lens. It is composed mainly of collagen type IV and laminin (2). The cortex consists of epithelial cells, which gradually turn into fibres. A single-layered lens epithelium is located anteriorly, under the lens capsule. Lens epithelium cells have their apical surface facing inwards towards the nucleus and the basal surface outward, touching the lens capsule. The lens epithelium is the first physical and biological barrier of the lens and the metabolically the most active part, maintaining the physiological balance. The ionic pump in the lens epithelial cells maintains homeostasis and clarity of the lens. The anterior lens epithelium also serves as a source of precursor cells, from which the fibre cells are formed by molecular and morphological differentiation.

The lens nucleus is composed of the oldest cells, virtually without metabolic activity (3). It consists of a mass of compressed fibre cells that are covered anteriorly with single-layer epithelium and wrapped with the lens capsule. The amount of the fibre cells provides the functional properties of the lens.

Figure 1: Lens structure schematic presentation.



2. The posterior capsule opacification (PCO) development

In cataract surgery (phacoemulsification), after removal of the anterior lens capsule together with lens epithelial cells measuring approximately 5.5 mm, the cortex and the nucleus of the natural eye lens are removed and a new artificial lens is inserted. The surgery is very safe and rarely associated with complications, however, relatively often posterior capsule opacification (PCO) occurs after the surgery. Upon the cataract surgery, the attenuation of visual acuity due to the PCO may develop in approximately 20 % of cases within a period of five years (4). PCO usually develops from the lens epithelial cells that remain on the lens capsule after cataract surgery (phacoemuslification) (4,5). During the PCO formation, rather than the posterior lens capsule itself becoming opaque, opaque secondary membranes develop on the posterior lens capsule (6-8). The secondary membranes are formed by proliferation, migration (9) and transformation (so-called epithelial-mesenchymal transition) of the lens epithelial cells (10,11). Thus modified cells are capable of depositing the collagen and restoring or regenerating lens fibres in the area between the artificial intraocular lens and the posterior lens capsule (12). Lens epithelial cells proliferate in various patterns. Clinically, two basic morphological forms of PCO are distinguished: fibrous and pearl. The first is formed by the proliferation and migration of the lens epithelial cells, which are located under the anterior lens capsule and the passage in myofibroblast (so-called epithelial-mesenchymal transition) (13). The second form, with the pearls, is formed from the lens epithelial cells,

which are located in the equatorial part of the lens or the lens pole and regenerate into the lens fibres, which express crystalline (5,14).

3. Lens regeneration or renewal

The latest research results show that the lens can renew–regenerate after a new way of congenital cataracts surgery, where the key is the lens epithelium. The new way of congenital cataracts surgery preserves larger part of lens epithelial cells, which then proliferate and form a functional lens. The surgery turned out to be successful in both rabbits and monkeys, as well as in children with congenital cataracts (15).

Cataract is one of the major causes of visual impairment in children (16). The present method of cataract surgery in children includes the creation of a large opening in the anterior lens capsule with a diameter of 6 mm, resulting in a large area of the removed capsule, and thus the removal of a large number of lens epithelial cells. In this way, the incidence of PCO is reduced. Recently, a new way of congenital cataract surgery has been described, in which the aperture of a smaller diameter is formed on the periphery of the anterior lens capsule (<1.5 mm) in order to enable lens regeneration. This leaves more lens epithelial cells in the lens capsular bag, which give rise to a new functional lens.

Twelve children with congenital cataract (24 eyes) had a new minimally invasive surgery which allows lens regeneration. The control group of 25 children with congenital cataract (50 eyes) had a standard cataract surgery (15). The new method of surgery has two advantages: it considerably reduces the size of the removed lens capsule and at the same time moves the opening of the lens capsule from the central visual axis to the periphery. After the surgery, the cornea, anterior chamber and fundus were clear. Complications associated with surgery have not been detected. Openings in the anterior lens capsule healed in one month. Renewed clear biconvex lens structure was formed three months after the surgery. After six months, no complications or unorganized tissue regeneration were recorded.

The new minimally invasive surgical method provides clear visual axis while preserving lens epithelial cells that have regenerative potential.

Lens epithelial cells in the adult human eyes show an increased regenerative potential after injury (15), which indicates the possibility of functional lense renewal also in older patients with age-related cataracts.

4. Preventing or promoting the growth of lens epithelial cells

In the new mode of congenital cataract surgery, lens epithelial cells are responsible for both PCO as well as the emergence of a new lens.

Study of the inhibitory mechanisms of the epithelial transition to mesenchyme is important to prevent the development of PCO. It has been demonstrated that transforming growth factor β, TGFβ, induces a transition of epithelium to mesenchyme, which is central in the formation of fibrous PCO, probably via the ERK / MAPK signalling pathway (chain of proteins in the cell that transmits a signal from the cell surface receptors to the DNA in the cell nucleus) (17). Fibronectin type III in the extracellular matrix protein tenascin-C plays a role in inhibiting the epithelial-mesenchymal transition (18).

On the other hand, the study of mechanisms of stimulating the differ-

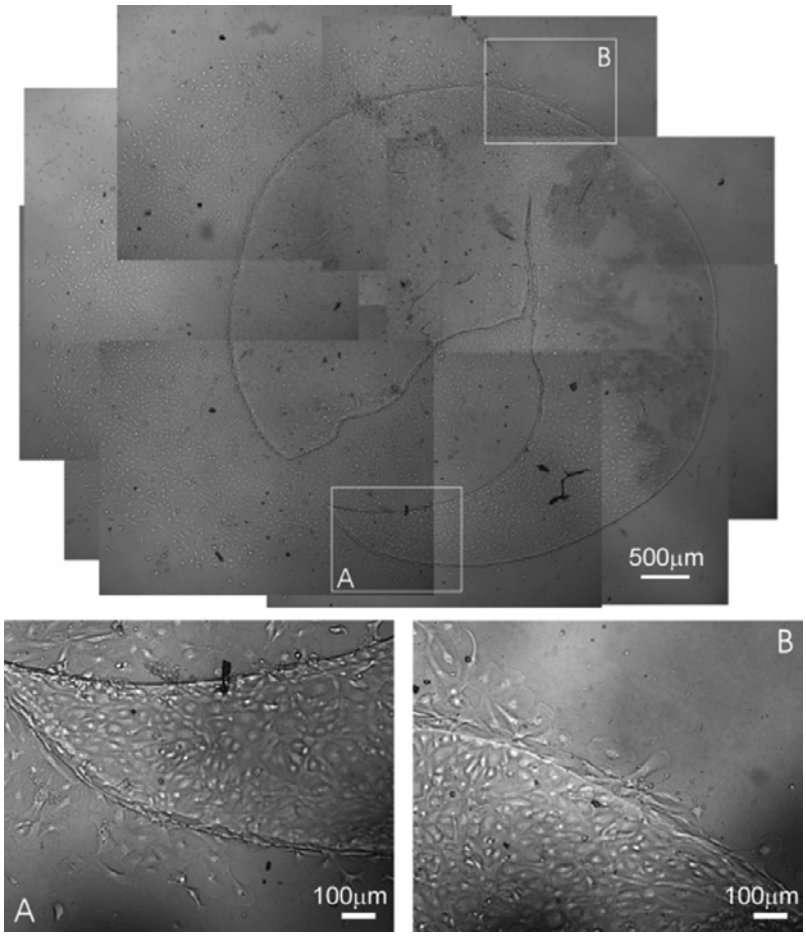


Figure 2: An example of an attached human anterior lens capsule explant with growing lens epithelial cells in a Petri dish. In the bottom panels, two areas of the lens capsule with growing epithelial cells are shown at larger magnification.

entiation of lens epithelial cells to fibre cells is important for the reconstruction of functional lens. Growth factors that act through tyrosine kinase receptors (RTKs), such as fibroblast growth factors (FGF), have normal regulatory role in the lens (19). Today it is known that the process of differentiation of lens epithelial cells to fibre cells is driven by FGF. Little is known about the factors that coordinate the detailed arrangements of fibre cells in functional lens. Recent research gives insight into FGF- activated mechanism that involves the interaction of Wnt-Frizzled and Jagged / Notch signalling pathways. Wnt and Notch signalling pathway are used by animal cells to control their identity and behaviour during development. Mutual interaction of lens epithelial cells and fibre cells is crucial for merging and maintaining a regulated three-dimensional structure, which is central to the functioning of the lens (17).

Study of lens epithelial cells proliferation is important as regarding the development of PCO the proliferation should be prevented, while regarding the formation of a new lens the proliferation should be stimulated. The proliferation grade of lens epithelial cells is age-dependent. In humans, lens epithelial cells have decreased responsiveness to mitogenic growth factor, FGF-2 (20). This is consistent with the drop in the density of human lens epithelial cells in the central area of the lens epithelium with age (21). Ki-67 nuclear protein is a cell marker associated with cell proliferation. Some of the Ki-67-positive cells were also detected in the central, anterior region of the epithelium in young bovine and human lens, where a younger lens has more proliferating cells than an older one (22). Lens epithelial cells proliferation was studied in the adult mice lens epithelium, where they found two groups of cells: slower, rarely cycling cells, which are located in the central area of lens epithelium, and more frequently cycling cells in the peripheral or germinative area (23). There is a relatively low percentage of the first cells (2–3 %), so the authors suggest that these are lens epithelial stem cells, which rarely divide during homeostasis. With the disturbance, these cells start proliferating and provide the division, which will supply the central and germinate area with the cells that have the capability of further division. The interaction of the apical surfaces of lens epithelial cells, which are facing inwardly towards the nucleus, with the inner portion of the lens, where they contact the apical surfaces of the fibre cells, regulates

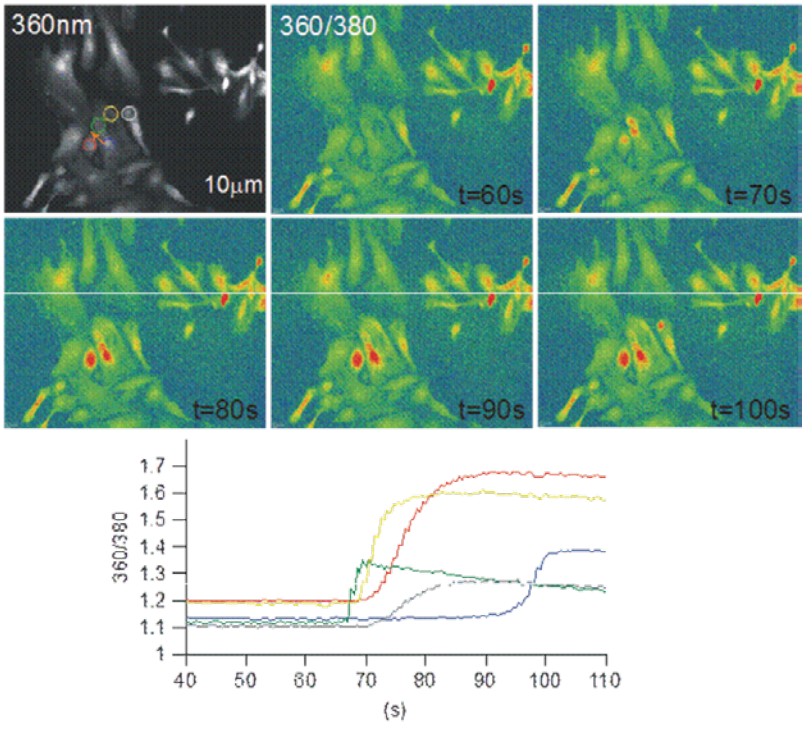


Figure 3: An example of changes in the intracellular calcium concentration of the lens epithelial cells grown in culture of lens capsule explant, in response to mechanical stimulation. The stimulation site is indicated by an arrow in the first picture, which shows the cell morphology at 360 nm. Time changes in intracellular calcium concentrations for 5 selected areas are shown below; the areas are marked with circles of the same colour. Colour images through 360/380 nm show the rise in calcium concentration at selected moments: red colour shows the highest concentration, yellow smaller and green the smallest.

lens epithelial cells proliferation (24,25). BMI-1, a protein which in humans is encoded by the BMI-1 gene (B cell-specific murine leukemia virus), is necessary for the maintenance and renewal of the endogenous lens epithelial cells. Loss of the BMI-1 leads to a decrease in the proliferative capacity of lens epithelial cells and the formation of cataracts (15).

5. The methods of analysis of lens epithelial cells after cataract surgery at our laboratory

To study the role of the human lens epithelium, both its regenerative potential and the PCO development, the anterior lens capsule obtained from cataract surgery can be used (26), to grow ex vivo explant cultures of the lens capsule (27,28). At the Eye Clinic Ljubljana we use anterior lens capsules obtained via standard cataract surgery, which are normally discarded. The lens epithelium structure and differences of apical and basal side we have shown using scanning and transmission electron microscopy and confocal microscope. With each of the three methods used we have shown the same morphological features, the extensions and the entanglements of the cytoplasmic membrane of lens epithelial cells at the border with the lens capsule, while it has been shown that the apical surface of lens epithelial cells is smooth (29). Using scanning and transmission electron microscopy, we studied the lens epithelium in patients with different cataract types. In patients with intumescent cataracts the lens epithelium has the following features: swollen cells, spherical formations and degraded cells (30). In patients with retinitis pigmentosa we observed the following features of the lens epithelium, such as holes and the degradation of the epithelium measuring from <1 μm to more than 50 μm (31). The holes in the lens epithelium likely have a role in the cataract development. Homeostasis of the intracellular calcium (Ca^{2+}) concentration is a general indicator of the functioning of cells. In the study of the role of the lens epithelium and the analysis of the intra- and intercellular Ca^{2+} signaling in human anterior lens epithelial cells we showed that in more developed cataracts, cells were found to exhibit a slower collective response to stimulation and a less pronounced spatio-temporal clustering of cells. The intercellular networks were found to be sparser and more segregated than in mild cataracts (32). We have also shown the contraction of lens epithelial cells upon nonspecific stimulation,

which is at least partly independent of the changes in intracellular Ca^{2+} concentration. Contraction can be induced by a mechanical stimulus, the response is fast and after the cessation of stimulation, cells tend to return to the initial non-contracted state (33). This contraction of cells 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.

In connection with the development of PCO and lens regeneration, we have shown that the anterior lens epithelium of an adult has pluripotent lens epithelial cells. Cultured human anterior lens capsule harbours lens epithelial cells that can proliferate and migrate, suggesting their pluripotency or putative stem cell nature. By immunostaining, we have shown that the Ki-67 nuclear protein that is associated with cell proliferation, and Sox2 transcription factor that is essential for pluripotency of undifferentiated embryonic stem cells, are expressed in the lens epithelial cells grown on the human anterior lens capsule (27). Stem and progenitor cells expressing Sox2 are important for tissue regeneration and survival in mice (34).

Anterior lens capsules obtained via cataract surgery we also use for the cultivation of ex vivo explants of the lens capsule by plating the lens capsule in a Petri dish. For lens epithelial cells to migrate to the glass Petri dishes, the lens capsule must be attached to the bottom of the Petri dish. We have developed a method for attaching the explants using viscoelastic (28). An example of the attached human anterior lens capsule explant with growing lens epithelial cells is shown in Figure 2. Ex-vivo cultured explant of the lens capsule with the growing lens epithelial cells can serve as a model for testing a variety of physical and pharmacological effects. We have shown that targeted and localized microplasma causes dose-dependent morphological changes of cells and apoptosis of ex vivo cultured human lens epithelial cells. The results show that the single cell specific micropipette plasma can be used to selectively induce death in lens epithelial cells which remain in the capsular bag after cataract surgery and thus prevent their migration to the posterior lens capsule and PCO formation (35). The function of the growing lens epithelial cells of lens capsule explants can be analyzed by means of measurements which show changes in the intracellular Ca^{2+} concentration, which is an indicator of the cell functioning, as a response to a variety of physical and pharmacological stimuli. Figure 3 shows an example of changes in the intracellular Ca^{2+} concentration of the lens epithelial cells grown in culture from lens capsule explant, in response to mechanical stimulation, and shows intercellular Ca^{2+} signalling. The raise in Ca^{2+} concentration is not simultaneous in all the selected areas but shows a time delay, which indicates that the signal travels. The intercellular Ca^{2+} signalling indicates functional contacts between the cells that can be compared between the confluent and non-confluent lens epithelial cells. Similar studies are important for testing the pharmacological agents and studying the promotion and inhibition of lens epithelial cell growth.

6. Conclusions

The lens epithelial cells are responsible for both PCO as well as the lens regeneration after the new way of congenital cataract surgery. The new minimally invasive method of congenital cataract surgery in children preserves endogenous stem lens epithelial cells, thereby achieving functional lens regeneration.

The pluripotency of lens epithelial cells, which have the stem cell properties, is of key importance for the regeneration of the lens. Ex vivo cultures of human lens epithelial cells can serve as a model for testing various physical and pharmacological effects for promoting or inhibiting the cell growth. The functionality of cells and the responses to various stimulations can be studied by analysing the intra- and intercellular Ca^{2+} signalling. Stimulating the growth of lens epithelial cells is important for regeneration of the lens after congenital cataract surgery, while inhibiting the growth is important for preventing the development of PCO.

References

1. Fan X, Monnier VM, Whitson J. Lens glutathione homeostasis: Discrepancies and gaps in knowledge standing in the way of novel therapeutic approaches. Experimental Eye Research. 2016.
2. Danysh BP, Duncan MK. The lens capsule. Experimental Eye Research. 2009;88(2):151–64.
3. McNulty R, Wang H, Mathias RT, Ortwerth BJ, Truscott RJW, Bassnett S. Regulation of tissue oxygen levels in the mammalian lens. The Journal of Physiology. 2004;559(3):883–98.
4. Duncan G, Wang L, Neilson GJ, Wormstone IM. Lens Cell Survival after Exposure to Stress in the Closed Capsular Bag. Investigative Opthalmology & Visual Science. 2007;48(6):2701.
5. Apple DJ, Solomon KD, Tetz MR, Assia EI, Holland EY, Legler UFC, et al. Posterior capsule opacification. Survey of Ophthalmology. 1992;37(2):73–116.
6. Hiles DA, Johnson BL. The role of the crystalline lens epithelium in postpseudophakos membrane formation. American Intra-Ocular Implant Society Journal. 1980;6(2):141–7.
7. McDonnell PJ, Green WR, Maumenee AE, Iliff WJ. Pathology of Intraocular Lenses in 33 Eyes Examined Postmortem. Ophthalmology. 1983;90(4):386–403.
8. Roy FH. After-cataract: clinical and pathologic evaluation. Ann Ophthalmol. 1971;3(12):1364–6.
9. Lauffenburger DA, Horwitz AF. Cell Migration: A Physically Integrated Molecular Process. Cell. 1996;84(3):359–69.
10. Wormstone IM, Wang L, Liu CSC. Posterior capsule opacification. Experimental Eye Research. 2009;88(2):257–69.
11. Wormstone IM, Wang L, Liu CSC. Posterior capsule opacification. Experimental Eye Research. 2009;88(2):257–69.
12. Awasthi N. Posterior Capsular Opacification. Archives of Ophthalmology. 2009;127(4):555.
13. McDonnell PJ, Zarbin MA, Green WR. Posterior Capsule Opacification in Pseudophakic Eyes. Ophthalmology. 1983;90(12):1548–53.
14. Saika S. Relationship between posterior capsule opacification and intraocular lens biocompatibility. Progress in Retinal and Eye Research. 2004;23(3):283–305.
15. Lin H, Ouyang H, Zhu J, Huang S, Liu Z, Chen S, et al. Lens regeneration using endogenous stem cells with gain of visual function. Nature. 2016;531(7594):323–8.
16. Gogate P, Kalua K, Courtright P. Blindness in Childhood in Developing Countries: Time for a Reassessment? PLoS Medicine. 2009;6(12):e1000177.
17. Lovicu FJ, Shin EH, McAvoy JW. Fibrosis in the lens. Sprouty regulation of TGFβ-signaling prevents lens EMT leading to cataract. Experimental Eye Research. 2016;142:92–101.
18. Tiwari A, Ram J, Luthra-Guptasarma M. Targeting the fibronectin type III repeats in tenascin-C inhibits epithelial-mesenchymal transition in the context of posterior capsular opacification. Invest Ophthalmol Vis Sci. 2014;56(1):272–83.
19. Zhao G, Wojciechowski MC, Jee S, Boros J, McAvoy JW, Lovicu FJ. Negative regulation of TGFβ-induced lens epithelial to mesenchymal transition (EMT) by RTK antagonists. Experimental Eye Research. 2015;132:9–16.
20. Dawes LJ, Duncan G, Wormstone IM. Age-Related Differences in Signaling Efficiency of Human Lens Cells Underpin Differential Wound Healing Response Rates following Cataract Surgery. Investigative Opthalmology & Visual Science. 2013;54(1):333.
21. Guggenmoos-Holzmann I, Engel B, Henke V, Naumann GO. Cell density of human lens epithelium in women higher than in men. Invest. Ophthalmol Vis Sci. 1989;30(2):330–2.
22. Wu JJ, Wu W, Tholozan FM, Saunter CD, Girkin JM, Quinlan RA. A dimensionless ordered pull-through model of the mammalian lens epithelium evidences scaling across species and explains the age-dependent changes in cell density in the human lens. Journal of The Royal Society Interface. 2015;12(108):20150391.
23. Zhao G, Wojciechowski MC, Jee S, Boros J, McAvoy JW, Lovicu FJ. Negative regulation of TGFβ-induced lens epithelial to mesenchymal transition (EMT) by RTK antagonists. Experimental Eye Research. 2015;132:9–16.
24. Shi Y, De Maria A, Lubura S, iki H, Bassnett S. The Penny Pusher: A Cellular Model of Lens Growth. Investigative Ophthalmology & Visual Science. 2014;56(2):799–809.
25. Rakic J-M, Galand A, Vrensen GFJM. Separation of Fibres from the Capsule Enhances Mitotic Activity of Human Lens Epithelium. Experimental Eye Research. 1997;64(1):67–72.
26. Andjelić S, Zupančič G, Perovšek D, Robič T, Hawlina M. Anterior lens capsule as a tool to study the physiology of human lens epithelial cells. Zdrav

Vestn / Slovenian Medical Journal 2010;79:I-123–30.
27. Andjelić S, Drašlar K, Lumi X, Yan X, Graw J, Facskó A, et al. Morphological and proliferative studies on ex vivo cultured human anterior lens epithelial cells–relevance to capsular opacification. Acta Ophthalmologica. 2015;93(6):e499-e506.
28. Andjelic S, Lumi X, Veréb Z, Josifovska N, Facskó A, Hawlina M, et al. A Simple Method for Establishing Ad herent Ex Vivo Explant Cultures from Human Eye Pathologies for Use in Subsequent Calcium Imaging and Inflammatory Studies. Journal of Immunology Research. 2014;2014:1–10.
29. Andjelic S, Drašlar K, Hvala A, Lopic N, Strancar J, Hawlina M. Anterior lens epithelial cells attachment to the basal lamina. Acta Ophthalmologica. 2015;94(3):e183-e8.
30. Andjelic S, Drašlar K, Hvala A, Hawlina M. Anterior lens epithelium in intumescent white cataracts–scanning and transmission electron microscopy study. Graefe's Archive for Clinical and Experimental Ophthalmology. 2015;254(2):269–76.
31. Andjelic S, Draslar K, Hvala A, Hawlina M. Anterior lens epithelium in cataract patients with retinitis pigmentosa–scanning and transmission electron microscopy study. Acta Ophthalmologica. 2015;93:n/a-n/a.
32. Gosak M, Markovič R, Fajmut A, Marhl M, Hawlina M, Andjelić S. 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(12):e0143781.
33. Andjelić S, Zupančič G, Perovšek D, Hawlina M. Human anterior lens capsule epithelial cells contraction. Acta Ophthalmologica. 2011;89(8):e645–53.
34. Arnold K, Sarkar A, Yram Mary A, Polo Jose M, Bronson R, Sengupta S, et al. Sox2+ Adult Stem and Progenitor Cells Are Important for Tissue Regeneration and Survival of Mice. Cell Stem Cell. 2011;9(4):317–29.
35. Recek N, Andjelić S, Hojnik N, Filipič G, Lazović S, Vesel A, 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(11):e0165883.