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Morphological and proliferative studies on *ex vivo* cultured human anterior lens epithelial cells – relevance to capsular opacification

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ABSTRACT.

Purpose: **To determine the structural characteristics of lens epithelial cells (LECs) found on the anterior portion of the lens capsule and their pluripotency, proliferating and migrating potential when grown *ex vivo* with relevance to posterior capsular opacification (PCO) after cataract surgery.**

Methods: **The explants of anterior portion of the lens capsule consisting of monolayer of LECs were obtained from uneventful cataract surgery and were cultivated under adherent conditions. The size and shape of the outgrowing cells were recorded by scanning electron microscopy (SEM), while their migration and proliferation potential were followed using light microscopy. Positivity for proliferation (Ki-67)- and pluripotency (Sox2)-specific markers were tested by immunofluorescent staining.**

Results: **The proliferation and migration of anterior portion of the lens capsule's LECs filling up the denuded and reverse side regions of the lens capsule as well as their growth on glass culture surfaces could be followed by light microscopy and SEM, while the distribution of LECs and their morphology could be analysed in detail by SEM. The expression of Ki-67 and Sox2 in LECs growing adherently on human anterior portion of the lens capsule could also be detected.**

Conclusions: **Classic light microscopy and SEM can be used to show that human anterior portion of the lens capsule harbours LECs that can proliferate and migrate, suggesting their pluripotency or putative stem cell nature. Similarly, morphological techniques can be used to study PCO and the effect different drugs or physical treatments have against PCO development.**

Key words: lens epithelial cells – pluripotency – posterior capsular opacification proliferation – scanning electron microscopy

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Introduction

Cataract surgery and posterior capsular opacification development

Following cataract surgery, a secondary loss of acuity due to posterior capsular opacification (PCO) can develop in up to 20% of eyes within 5 years (Duncan et al. 2007). Extracapsular cataract extraction or phacoemulsification of the opaque lens fibres leaves behind a sac-like structure in the eye – the lens capsular bag. This bag consists of a proportion of the anterior and the entire posterior lens capsule (Allen & Vasavada 2006). PCO occurs due to a robust growth of residual lens epithelial cells (LECs) within the lens capsular bag (Duncan et al. 2007). Cell proliferation is one of the key factors involved in PCO formation, but in particular, LECs' migration (Lauffenburger & Horwitz 1996) is an essential phenomenon occurring within the capsular bag. Lens epithelial cells that still reside on the remaining anterior portion of the lens capsule, despite surgical trauma, begin to recolonize the denuded regions of the anterior portion of the lens capsule, encroach onto the intraocular lens (IOLs) surface and, most importantly, colonize the previously cell-free posterior lens capsule, ultimately obstructing the visual axis

and giving rise to light scatter (Wormstone et al. 2009). Although a thin layer of cells is insufficient to affect the light path, subsequent changes to the matrix and cell organization can give rise to such scatter. Lens epithelial cells have the ability to migrate by adhering and crawling onto extracellular and artificial surfaces such as that of IOLs (Goldmann 2012), playing an important role in PCO formation.

Proliferation and pluripotency of anterior LECs

There is ample evidence about the proliferative potential of LECs published over the last 50 years. Generally, the central, anterior epithelial region is considered to be quiescent, whereas the proliferation is mainly restricted to the germinative zone, anterior to the equator (Martinez & de Iongh 2010). Although equatorial LECs have been known to be pluripotent, it is still controversial whether anterior LECs are pluripotent as well. To be considered as pluripotent, the cells should be able to migrate, proliferate and differentiate. The differentiation potential of anterior LECs has already been studied (Zelenka et al. 2009; Qiu et al. 2012) and so has been the anterior LECs' migration *in vitro* in a capsular bag model (Dawes et al. 2012). However, not much evidence exists about the structural phenotype of the anterior LECs.

So far, lens capsule material from healthy and cataractous human lenses and rabbits has been studied structurally by scanning electron microscopy (SEM) (Los et al. 1989; Jongebloed et al. 1990). In addition, the degeneration of capsular epithelium of cataractous lenses has been studied by SEM (Jongebloed et al. 1993). The ultrastructure of the lens particles remaining in the eye after extracapsular lens extraction has been investigated in rabbits by SEM, where these remnants of the anterior portion of the lens capsule have been shown as lined by a monolayer of epithelial cells (Kappelhof et al. 1985).

As the pluripotency of anterior portion of the lens capsule's LECs is still controversial and their proliferation and migration in the capsular bag after cataract surgery can lead to development of PCO, we studied the proliferating and migrating potential and characterized structurally the *ex vivo* cultured LECs growing out of human anterior portion of the lens capsule. The structural studies were carried out by SEM and light microscopy, while the molecular characterization was carried out by immunofluorescent staining for proliferation and pluripotency markers on the *ex vivo* cultured LECs growing out of human anterior portion of the lens capsules.

Materials and Methods

Tissue collection and processing

All tissue collection complied with the Guidelines of the Helsinki Declaration and was approved by the National Medical Ethics Committee of Slovenia, and all patients signed an informed consent form before the operation. The lens capsule explants were obtained from routine uneventful cataract surgery performed at the Eye Hospital, University Medical Centre (UMC), Ljubljana, Slovenia (age: 61.7 ± 6.0, $n = 3$; *cataracta progrediens*). Lenses were dissected so that the anterior portion of the lens capsule (i.e. basal lamina and associated LECs) were isolated from the fibre cells that form the bulk of the lens. Primary human LEC cultures were established by plating adherently the intact human anterior portion of the lens capsules onto cell culture Petri dishes. After the surgery, the LEC capsules were transferred, one capsule specimen per dish, to cell culture plastic glass bottom Petri dishes (Mattek Corp., Ashland, MA, USA; 35 mm in diameter) and cultivated *ex vivo* under adherent conditions in high glucose medium (DMEM; Sigma, No. 5671, St. Louis, MO, USA) supplemented with 10% FBS and 1% antibiotics (penicillin–streptomycin; Sigma, No. 4333) in a CO_2 incubator (Innova CO-48; New Brunswick Scientific, Edison, NJ, USA) at 37°C and 5% CO_2. The Petri dish bottom central part with the diameter of 14 mm is made of glass and the rest is plastic. The Petri dish is poly-d-lysine coated, and the dish surface was not treated in any way. The preparations were cultured until epithelial cells had recolonized the cell-denuded areas of the lens capsule and had migrated from the lens capsule onto the dish as observed by light microscopy and subsequently by SEM.

Light microscopy

The proliferation and migration of the outgrowing cells were recorded and followed on a daily basis up to 8 days and throughout their continued growth using light microscope (Axiovert S100; Carl Zeiss, AG, Oberkochen, Germany). Image acquisition was carried out by a 12-bit cooled CCD camera SensiCam (PCO Imaging AG, Kelheim, Germany). The software used for the acquisition was WINFLUOR (written by J. Dempster, University of Strathclyde, Glasgow, UK). Microscope objectives used were as follows: 4×/0.10 Achroplan, 10×/ 0.30 Plan-NeoFluar and 40×/0.50 LD A-plan (Zeiss, Oberkochen, Germany).

Scanning electron microscopy

The anterior portion of the lens capsule explant cultures was grown for 20–22 days and then prepared for SEM. Culture medium was removed, washing step was applied to the specimens with sodium cacodylate buffer 0.1 M, pH 7.2, and then the specimens were double fixed: first, by 1% glutaraldehyde and 0.5% formaldehyde in 0.1 M cacodylate buffer, pH 7.2 for 2 hr (25% glutaraldehyde EM grade; SPI and formalin were obtained from paraformaldehyde; Sigma); second, by 1% OsO_4 in cacodylate buffer for 45 min. Dehydration of the lens capsule tissue was performed in an ethanol cascade. For drying of specimens, critical point drying (CPD, CPD 030 Critical Point Dryer, Balzers Union AG, Balzers, Liechtenstein (now Leica)) procedure with CO_2 was applied. Dried specimens were glued by carbon adhesive discs to specimen stubs, then Pt sputtered (Bal-Tec SCD 050 Sputter Coater, Balzers Union AG) and examined in field emission scanning electron microscope (FESEM, 7500F; JEOL, JSM, Tokyo, Japan).

Immunofluorescent staining

Anterior lens capsules obtained freshly after cataract surgery were fixed in 4% paraformaldehyde for 20 min, room temperature. The samples were dehydrated and embedded in paraffin after which 3-μm-thick longitudinal sections were obtained for immunofluorescent labelling with anti-Ki-67 (rb, 1:200; Neo Markers) and Sox2 (rb, 1:500;

Chemicon) antibodies and counterstained with a nuclear stain 4′,6-Diamidino-2-Phenylindole (DAPI). Negative controls were stained with secondary antibodies only. For visualization, ZEISS Axio Observer.Z1 (Zeiss) fluorescent microscope was used. The analysis was carried out on 3 anterior portions of the lens capsules and was repeated on at least five sections per donor obtained independently.

Results

Light microscopy of the cultured human anterior portion of the lens capsule's LECs proliferation and migration

Primary human LEC cultures were established by plating adherently the intact human aLCs onto cell culture Petri dishes. The preparations were cultured until epithelial cells had recolonized the cell-denuded areas of the LC and had migrated from the LC onto the dish as observed by light microscopy and subsequently by SEM. The proliferation and migration potential of the LECs were followed on a daily basis throughout their continued growth using light microscopy (Fig. 1). At 24 hr postplating (Fig. 1A), a healthy lens epithelium adherent to the lens capsule and filling up the cell-denuded region of the lens capsule could be observed. At day 3 (Fig. 1B), the cells that migrated to the denuded region of the lens capsule and to the Petri dish could also be observed. By day 4 (Fig. 1C), the cells had recolonized the denuded regions of the lens capsule and started migrating out of the lens capsule and onto the glass surface of the culture Petri dish. At day 8, the cells started migrating to the opposite side of the lens capsule observed using a different plane focus during microscopy (not shown here). Figure 1 provides information about the migrational velocity of the cells and the distance they are able to transpass.

Scanning electron microscopy of the human anterior portion of the lens capsule obtained freshly after cataract surgery

The presence and appearance of cell-denuded regions on the non-cultured human anterior portion of the lens capsule obtained freshly after cataract surgery was confirmed by SEM as shown in Fig. 2. In general, the cell nuclei protruded from the undersurface in the region close to the denuded area. The mosaic pattern of the non-cultured anterior portion of the lens capsule's LECs obtained freshly after cataract surgery was regular with the cuboidal LECs having an indented nuclei arranged in a monolayer on the underlying anterior portion of the lens capsule. Figure 3D shows LECs with more prominent microvilli on their apical surface, while Figs 2 and 3 show the appearance of anterior portion of the lens capsule explant with the LECs on it before proliferating and migrating.

Scanning electron microscopy of the cultured human anterior portion of the lens capsule explant LECs

To exclude the possibility that the LECs growing on the opposite side of the denuded region of the human anterior portion of the lens capsule were not LECs growing on the glass surface of the Petri dish, SEM of the cultured human anterior portion of the lens capsule explant was performed (Fig. 4). The SEM confirmed that the LECs grow on both sides of the human anterior portion of the lens capsule when placed in a culture dish. Their growth was imaged after 22 days of culture. The rolled-up region represents the inner (concave) side of the anterior

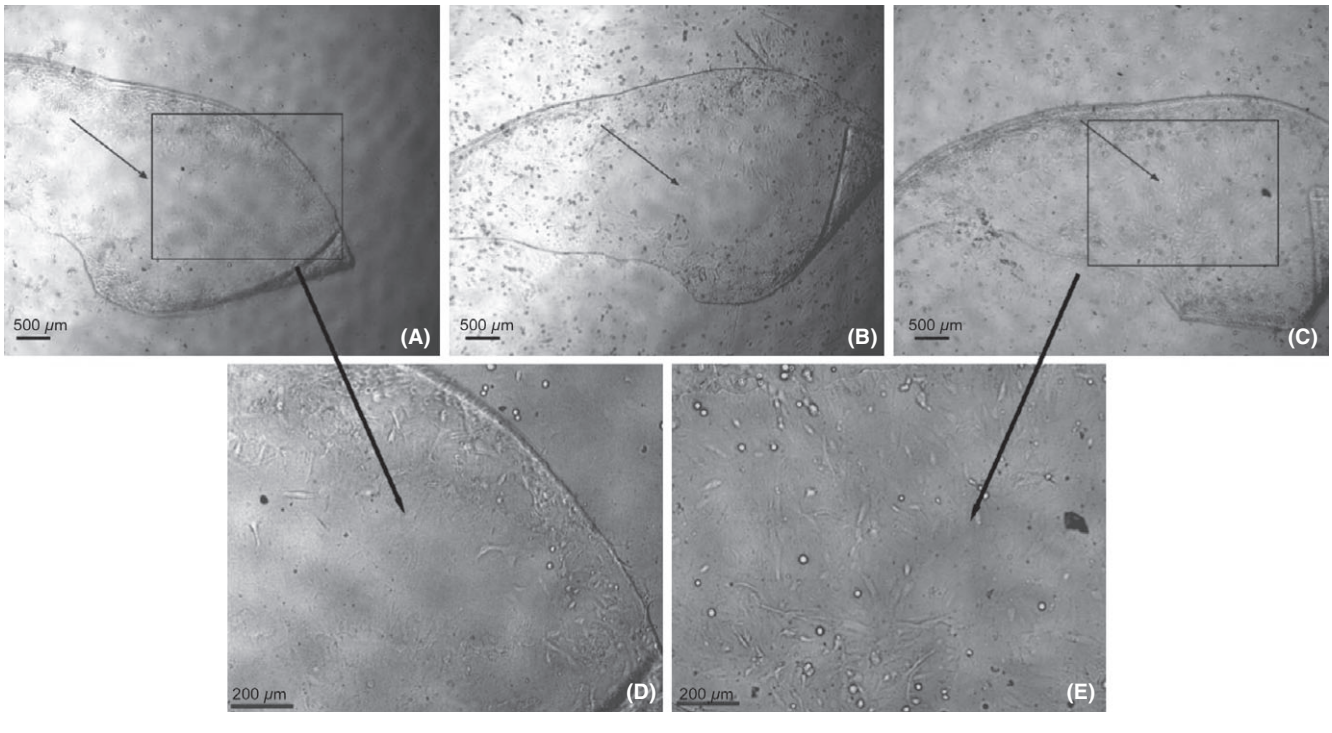

Fig. 1. Lens epithelial cells (LECs') growth on cultured human anterior portion of the lens capsule explant and onto a Petri dish followed by light microscopy. Cell cultures at day 1 (A), day 3 (B) and day 4 (C) are shown. The arrows point at enlarged inserts (D) and (E) showing a cell-denuded region of the anterior portion of the lens capsule that with time becomes recolonized by LECs.

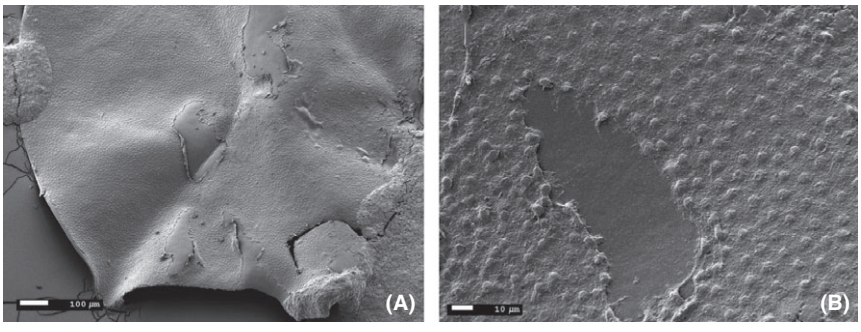

Fig. 2. Example showing the cell-denuded regions on the freshly isolated, non-cultured human anterior portion of the lens capsule, as imaged by scanning electron microscopy (parts (A) and (B) are different magnifications and views of the same sample; scale bar is appropriately marked in each case).

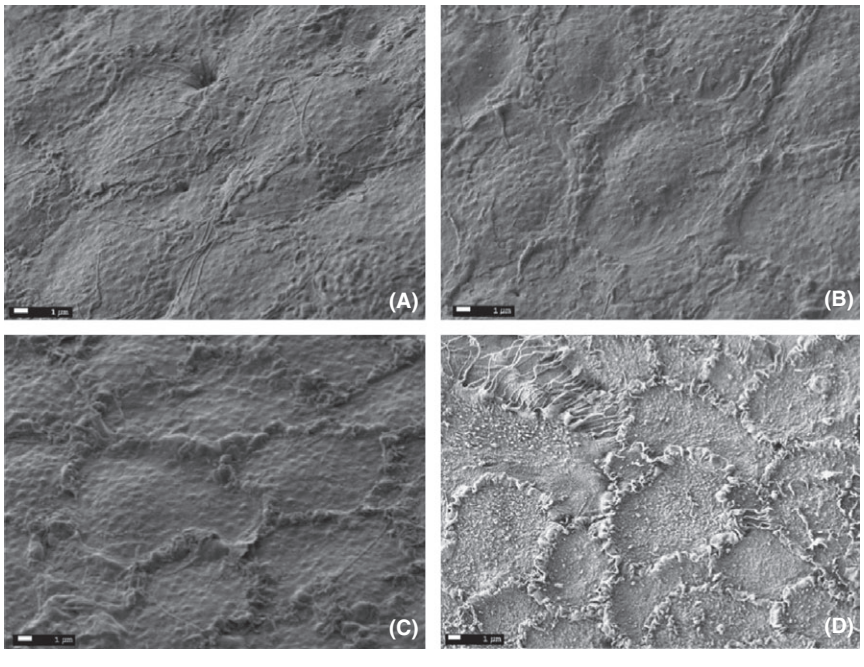

Fig. 3. Non-cultured anterior portion of the lens capsule-lens epithelial cells obtained freshly after cataract surgery showing intercellular connections (A–C) and more prominent microvilli on their apical surface.

portion of the lens capsule containing a monolayer of LECs, which in this case was covered by degenerating cell material. The flat region presents the outer (convex) side of the lens capsule that is smooth and without the cells in the intact lens. In cultured anterior portion of the lens capsule, the growth of the LECs on the anterior portion of the lens capsule outer side could be observed. Figure 4C shows the halos these LECs form.

The LECs that over time migrated out of the anterior portion of the lens capsule explant onto the surface of the cell culture Petri dish were also imaged by SEM. The distribution of these LECs and their morphology in the regions with the non-confluent and confluent LECs was analysed in details after 20 days in culture. The LECs that were not yet confluent remained attached to the glass bottom and assumed a microvillar-like morphology and intercellular connections by filaments (Fig. 5). The LECs surrounding halos and protrusions were also visible, while some cells were noticed for having more prominent microvilli on their apical surface than others.

Figure 6 shows the LECs which migrated into the non-confluent and the confluent culture. Cell migration began with protrusion of the cell membrane followed by the formation of new adhesions at the cell front that linked the cytoskeleton to the substratum, which in the case of non-confluent culture could be the denuded region of the human anterior portion of the lens capsule or the glass surface of the cell culture Petri dish (Fig. 5A), while in the case of the confluent culture was the apical surface of the adjacent LECs of the newly created lens epithelium (Fig. 5B). Lamellipodia, which are projections on the mobile edge of the cell, and filopodia that spread beyond the lamellipodium frontier could also be seen here.

When the cells reached confluence, mosaic pattern of LECs could be visible with the cells forming close connections with each other supported by filaments (Fig. 7). Small holes or empty areas could still be seen in some regions between the cells. The mosaic pattern was not regular. Confluent human anterior LECs were not strongly con-

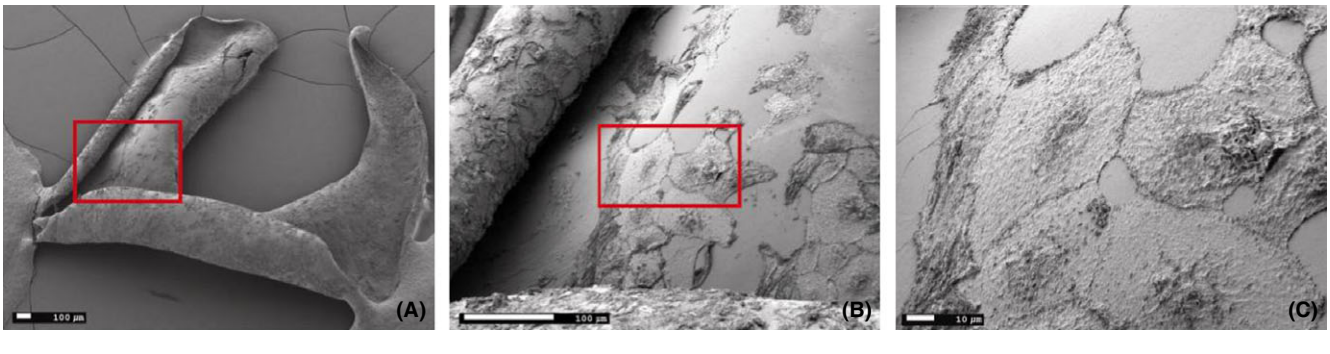

Fig. 4. Scanning electron microscopy showing lens epithelial cells on both sides of a cultured human anterior portion of the lens capsule explant. Parts (B) and (C) are enlarged images in a sequence from part (A) (scale bar is appropriately marked in each case).

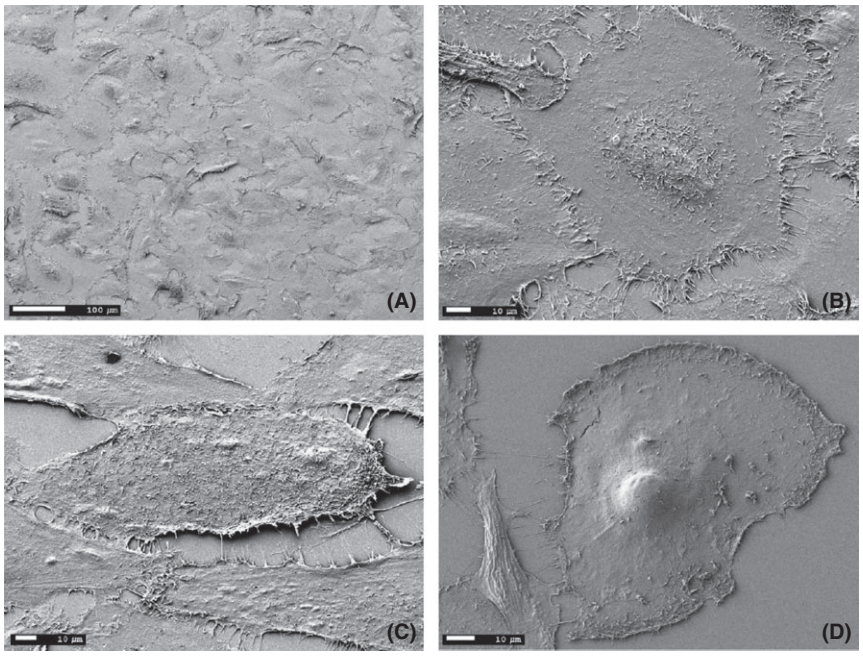

Fig. 5. Non-confluent (A, C, D) and confluent (B) lens epithelial cells growing onto the glass surface of a cell culture Petri dish. Lamellipodia formation is also visible (B–D) (scale bar is appropriately marked in each case).

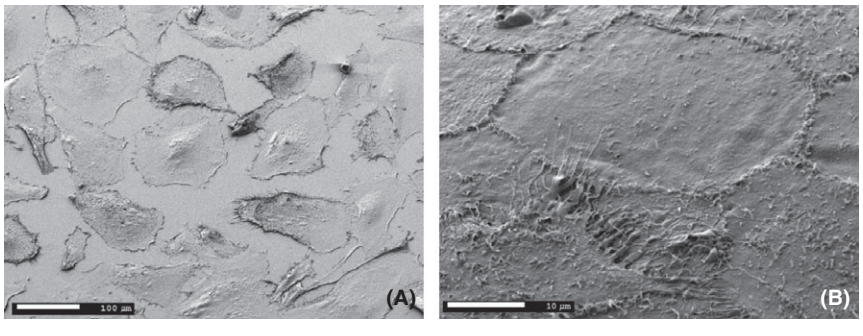

Fig. 6. Lens epithelial cells migrating into the non-confluent (A) and the confluent (B) culture (scale bar is appropriately marked in each case).

nected to the glass anymore, so that a monolayer of cultured LECs could detach from the glass and could be seen as a layer, implying it did not need adhesion support any further.

The lateral membranes of the LECs (membranes in contact with the adjacent epithelial cells) were highly tortuous. On Fig. 8, not only the filaments which were located on the apical cell surface connecting the adjacent confluent cultured human LECs from anterior portion of the lens capsule could be seen, but also the filaments located deeper towards the basal cell surface, on the lateral sides of the membranes, connecting the adjacent cells. At the apical cell surface, at fine resolution (Fig. 7C,D), small vacuoles became visible as well.

The filaments that were seen at the junction between the cultured human LECs from anterior portion of the lens capsule were similar to what could be observed at the junctions between the cells in the case of LECs in freshly isolated anterior portion of the lens capsules obtained after uneventful cataract surgery, suggesting they were similar to the natural connections existing between the cells in the anterior portion of the lens capsule (Fig. 3), and they were not only hexagonal imprints of the lens fibres.

Immunofluorescent staining for proliferation and pluripotency markers in the human anterior portion of the lens capsule obtained freshly after cataract surgery

Finally, the freshly isolated human anterior portion of the lens capsule-LECs could be characterized as positive for the proliferation (Ki-67) and pluripotency (Sox2) markers using immunofluorescent staining (Fig. 9).

Discussion

Using cultured human anterior portion of the lens capsule explants and visualizations by light microscopy, SEM and immunofluorescence staining for proliferation and pluripotency markers, we have shown that human anterior portion of the lens capsule contains LECs that can migrate and proliferate, suggesting that not only equatorial LECs can do so, but also anterior LECs. The anterior portion of the lens capsule explants can be used to study PCO development and its prevention, while SEM is still a very useful tool for such analysis.

When placed in culture, the anterior portion of the lens capsule explants consisting of monolayer of LECs could migrate and proliferate to fill up the cell-denuded areas of the anterior portion of the lens capsule on the same side of the capsule. These cells could also migrate to the opposite side of the anterior portion of the lens capsule as shown by both light microscopy and in detail by SEM. The proliferation and migration of LECs on glass culture surface could also be shown by both visualization techniques, while the distribution of the LECs and their morphology could be analysed in detail by SEM.

The fact that the LECs could migrate to the other side of the anterior portion of the lens capsule has also been observed by others in an *in vitro* capsular bag model (Dawes et al. 2012), where it was suggested that the changes on the cells on the outside of the anterior portion of the lens capsule can lead to anterior portion of the lens capsule fibrosis, which can affect intraocular lens positioning and reduced visual quality. Phase-contrast microscopy of cultured human capsular bag has shown LEC forming a confluent monolayer at day 20 (Li et al. 2008). In anterior portion of the lens capsule explant cultures, the lens epithelium had expanded to 70–80% confluence and possessed morphology consistent with the epithelial origin of the cells by day 10 (Yang et al. 2010).

Our studies are in agreement with the previous ones and also with the

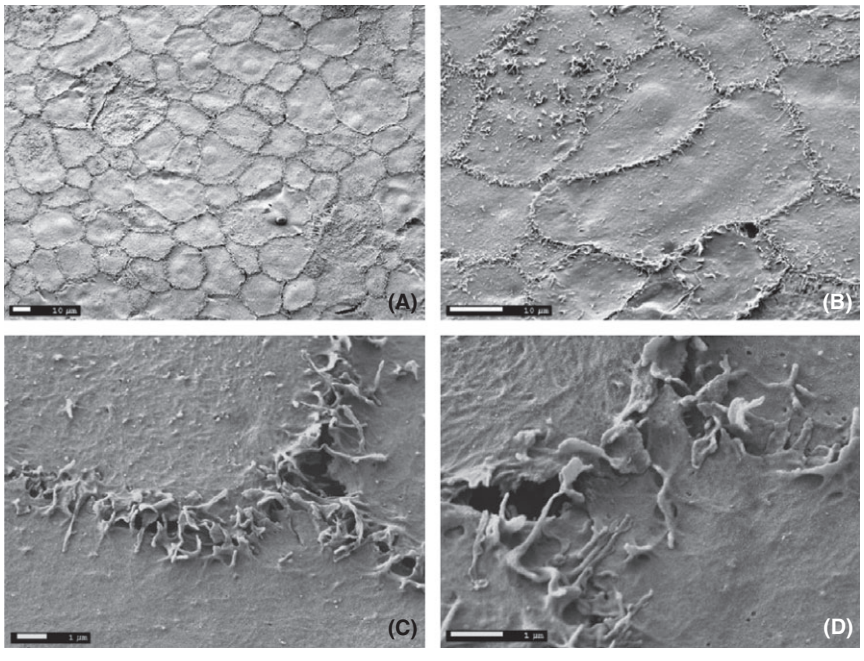

Fig. 7. Confluent lens epithelial cells grown on the surface of glass Petri dish showing lateral connections at different magnifications and views (scale bar is appropriately marked in each case).

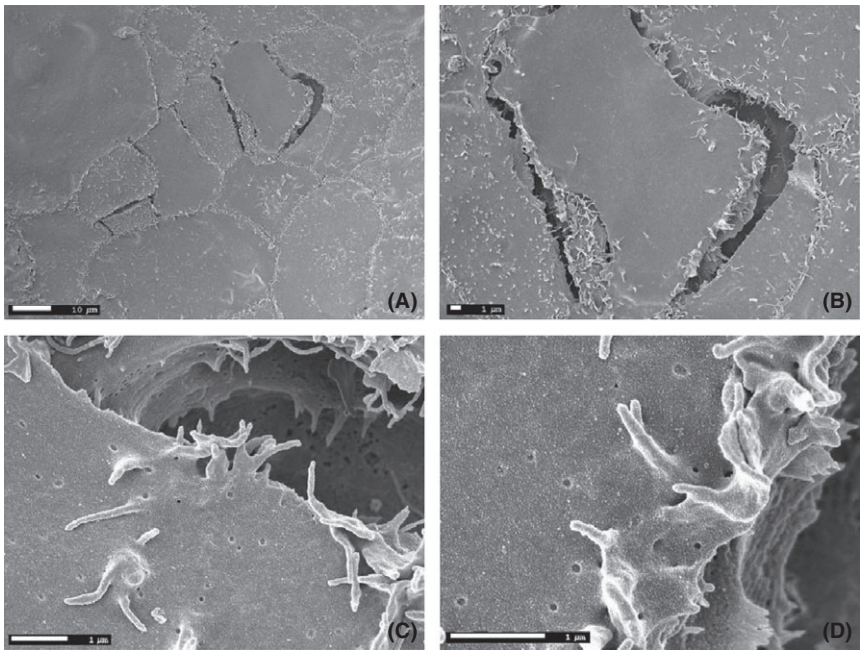

Fig. 8. Confluent lens epithelial cells grown on the surface of glass Petri dish showing deeper lateral connections from different perspectives of view (scale bar is appropriately marked in each case).

clinical data showing that the time needed for the LECs to migrate posteriorly is within 1–2 weeks after the surgery. The dimension of the surface that LECs pass in the eye from anterior to posterior lens capsule is comparable to the dimension they pass in anterior portion of the lens capsule explant culture, both towards the denuded areas of anterior portion of the lens capsules and the Petri dish surface.

Using SEM, we have provided more detailed information about the corresponding behaviour, distribution and morphology of cultured LECs. We have also shown that the cultured LECs become confluent and maintain morphological features similar to those of the LECs from the anterior portion of the lens capsule obtained freshly after cataract surgery. We have analysed the non-confluent and confluent LECs' grown both on anterior portion of the lens capsule and Petri dish by SEM after 20–22 days of culturing, and we could show the intercellular connections formed during the process of confluent LECs' culture monolayer formation. Furthermore, we could see the morphological features of the moving cells and distinguish lamellipodia and filopodia, cell halos and protrusions.

Filopodia play a central role in steering transient topographic preferences (Albuschies & Vogel 2013). The closure of epithelial gaps in the absence of cell injury is shown to be governed by the collective migration of cells through the activation of lamellipodium protrusions (Anon et al. 2012). Cell spreading represents the first step of cell motion involving different alterations in the cell's shape. We demonstrate that SEM is a good tool for detecting them. Appearance of lamellipodia and LECs spreading are the steps towards LECs' migration that occur in recolonization of denuded regions of the anterior portion of the lens capsule and also in migration of the LECs to the other side of the anterior portion of the lens capsule.

The lateral membranes of epithelial cells (membranes in contact with the adjacent epithelial cells) are highly tortuous (Dai & Boulton 2008). We show in detail their appearance on the LECs forming the confluent monolayer by SEM. The LECs' intercellular connections and the way of attachments to adjacent cells have been reviewed in Andjelic et al. (2011a). They are involved in LEC contractions as well (Andjelic et al. 2011b).

The Ki-67 protein which is a cellular marker strictly associated with cell proliferation was minimally expressed on LECs grown on human anterior portion of the lens capsule. Sox2-positive adult stem and progenitor cells have been shown to be important for tissue regeneration and survival in mice (Arnold et al. 2011). We show that Sox2 expression is also found in LECs grown on human anterior portion of the lens capsule. Therefore, using immunostaining, we could confirm the SEM and light microscopy findings

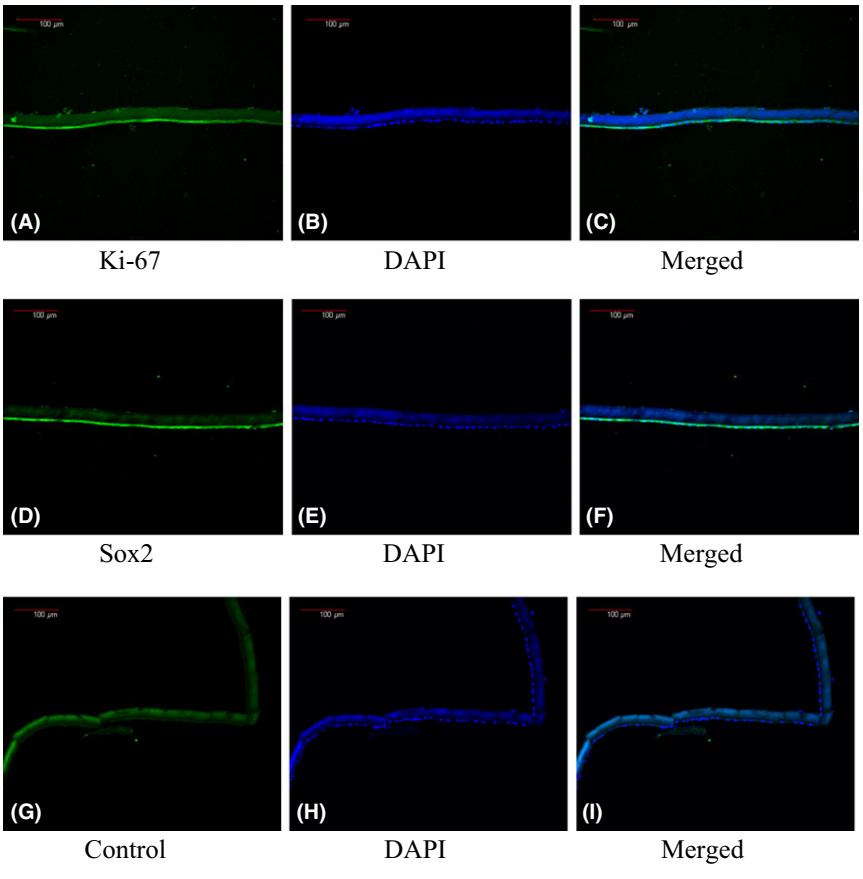

Fig. 9. Expression of proliferation- and pluripotency-specific markers in lens epithelial cells (LECs) grown on human anterior portion of the lens capsule shown by fluorescence microscopy. Immunohistochemistry was performed to detect the expression of Ki-67 (A–C) and Sox2 (D–F) in the LECs grown on human anterior portion of the lens capsule (left column: immunofluorescence for Ki-67 and Sox2; centre: nuclear, 4′,6-diamidino-2-phenylindole (DAPI) staining; right column: merged images; colours on the text correspond to the colour of the marker examined, while all nuclei are stained blue with DAPI; the images are representative of three anterior portions of the lens capsules being analysed and at least five sections per donor repeated independently (control staining (G–I) scale bar is appropriately marked in each case).

that the human aLECs can actually proliferate and are pluripotent.

This study gives new clues in a still controversial field of the pluripotency of aLECs. It indicates that proliferative LECs are also centrally located when surgically extracted human anterior portion of the lens capsule is studied. It is frequently reported that the centrally located LECs do not normally undergo mitosis (Colitz et al. 1999; Bhat 2001; Collison & Duncan 2001; Nguyen et al. 2003; Tamiya & Delamere 2005). Anterior LECs, if they replicate under normal circumstances, do so infrequently (Papaconstantinou 1967; Maisel et al. 1981; Piatigorsky 1981).

To date, no specific single molecular marker has been shown to exclusively identify lens stem cells or a lens stem cell niche (Martinez & de Iongh 2010). Side population cells which are smaller than other LECs are predominantly localized in and near the germinal zone and express higher levels of several known stem cell marker genes (Oka et al. 2010).

The development of PCO resulting from LECs' growth is essentially a wound healing response. Tissue formation during wound healing requires also an orchestrated movement of cells in particular directions or to specific locations, and this is what we have followed in the present study.

We would like to emphasize the SEM potential for LECs studies. Scanning electron microscopy can be used as a very powerful tool for following the LECs' migration, proliferation and differentiation which is of particular interest for the studies of anterior capsule LECs' pluripotency. Understanding the anterior LECs' migration and proliferation may help us develop better therapeutic strategies to prevent PCO, as well as study damage, in particular UV-based, *ex vivo* (Meyer et al. 2013; Øsnes-Ringen et al. 2013). Scanning electron microscopy can clearly show the changes in the LECs' structure, connections and distribution. Cell–cell junctions are critical for maintaining epithelial integrity and are increasingly becoming recognized as major centres of signalling that impact not only upon cell structure but also upon cell responses such as proliferation and differentiation (Goodwin & Yap 2004; Yap et al. 2007; Martinez & de Iongh 2010). Scanning electron microscopy can help answer the question how the intercellular communications are involved in the signalling necessary for proliferation and migration. LEC growth on the IOL surface is material dependent (Schild et al. 2005), and we suggest it can be studied by SEM as well. The latter is a powerful tool for studying the LECs association with the barrier effect as well as the morphological features of early migration of LECs (1–2 weeks postcataract) on the posterior lens capsule and their comparison to LECs found on the posterior lens capsule 2–3 years following surgery, when the PCO has fully formed. Scanning electron microscopy can also be used to associate specific LECs' morphological features with determined pathological types, in particular, anterior portion of the lens capsules obtained after different types of cataract surgery.

Anterior LEC culture may serve as a model for testing different pharmacological agents against PCO development, as the tissue is readily available after surgery and the studies are on human primary cells. Due to anterior LECs' pluripotency, proliferative and migration potential, their culturing can be used in future wound healing studies. Furthermore, biological treatment of cataract by introducing cultured LECs into cataractous lenses or by inducing transdifferentiation into other cell types can possibly be used for treatment of some other diseases as well.

References

Albuschies J & Vogel V (2013): The role of filopodia in the recognition of nanotopographies. Sci Rep **3**: 1658.

Allen D & Vasavada A (2006): Cataract and surgery for cataract. BMJ **333**: 128–132.

Andjelic S, Zupancic G & Hawlina M (2011a): The preparations used to study calcium in lens epithelial cells and its role in cataract formation. J Clin Exp Ophthalmol **S1**: 002. doi: 10.4172/2155-9570.S1-002.

Andjelic S, Zupancic G, Perovsek D & Hawlina M (2011b): Human anterior lens capsule epithelial cells contraction. Acta Ophthalmol **89**: 645–653.

Anon E, Serra-Picamal X, Hersen P, Gauthier NC, Sheetz MP, Trepat X & Ladoux B (2012): Cell crawling mediates collective cell migration to close undamaged epithelial gaps. Proc Natl Acad Sci USA **109**: 10891–10896.

Arnold K, Sarkar A, Yram MA et al. (2011): Sox2(+) adult stem and progenitor cells are important for tissue regeneration and survival of mice. Cell Stem Cell **9**: 317–329.

Bhat SP (2001): The ocular lens epithelium. Biosci Rep **21**: 537–563.

Colitz CM, Davidson MG & Mc GM (1999): Telomerase activity in lens epithelial cells of normal and cataractous lenses. Exp Eye Res **69**: 641–649.

Collison DJ & Duncan G (2001): Regional differences in functional receptor distribution and calcium mobilization in the intact human lens. Invest Ophthalmol Vis Sci **42**: 2355–2363.

Dai E & Boulton ME (2008): Basic Science of the lens. In: Yanoff M, Duker JS (eds). Ophthalmology. Edinburgh: Mosby/Elselvier 381–393

Dawes LJ, Illingworth CD & Wormstone IM (2012): A fully human in vitro capsular bag model to permit intraocular lens evaluation. Invest Ophthalmol Vis Sci **53**: 23–29.

Duncan G, Wang L, Neilson GJ & Wormstone IM (2007): Lens cell survival after exposure to stress in the closed capsular bag. Invest Ophthalmol Vis Sci **48**: 2701–2707.

Goldmann WH (2012): Mechanotransduction in cells. Cell Biol Int **36**: 567–570.

Goodwin M & Yap AS (2004): Classical cadherin adhesion molecules: coordinating cell adhesion, signalling and the cytoskeleton. J Mol Histol **35**: 839–844.

Jongebloed WL, Los LI, Dijk F & Worst JG (1990): Morphology of donor lens-capsule material studied by SEM. Doc Ophthalmol **75**: 343–350.

Jongebloed WL, Kalicharan D & Worst JG (1993): Human capsule epithelial cell degeneration, A LM, SEM and TEM investigation. Doc Ophthalmol **85**: 67–75.

Kappelhof JP, Vrensen GF, Vester CA, Pameyer JH, de Jong PT & Willekens BL (1985): The ring of Soemmerring in the rabbit: a scanning electron microscopic study. Graefes Arch Clin Exp Ophthalmol **223**: 111–120.

Lauffenburger DA & Horwitz AF (1996): Cell migration: a physically integrated molecular process. Cell **84**: 359–369.

Li JH, Wang NL & Wang JJ (2008): Expression of matrix metalloproteinases of human lens epithelial cells in the cultured lens capsule bag. Eye **22**: 439–444.

Los LI, Jongebloed WL & Worst JG (1989): Lens-capsule material of human and animal origin, studied by SEM. Doc Ophthalmol **72**: 357–365.

Maisel H, Harding CV, Alcalá JR, Kuszak JR & Bradley R (1981): The morphology of the lens. In: Bloemezrndal H (ed.). Molecular and cellular biology of the eye lens. New York, NY: Wiley and Sons 49–84

Martinez G & de Iongh RU (2010): The lens epithelium in ocular health and disease. Int J Biochem Cell Biol **42**: 1945–1963.

Meyer LM, Löfgren S, Holz FG, Wegener A & Söderberg P (2013): Bilateral cataract induced by unilateral UVR-B exposure – evidence for an inflammatory response. Acta Ophthalmol **91**: 236–242.

Nguyen MM, Nguyen ML, Caruana G, Bernstein A, Lambert PF & Griep AE (2003): Requirement of PDZ-containing proteins for cell cycle regulation and differentiation in the mouse lens epithelium. Mol Cell Biol **23**: 8970–8981.

Oka M, Toyoda C, Kaneko Y, Nakazawa Y, Aizu-Yokota E & Takehana M (2010): Characterization and localization of side population cells in the lens. Mol Vis **16**: 945–953.

Øsnes-Ringen O, Azqueta AO, Moe MC, Zetterström C, Røger M, Nicolaissen B & Collins AR (2013): DNA damage in lens epithelium of cataract patients in vivo and ex vivo. Acta Ophthalmol **91**: 652–656.

Papaconstantinou J (1967): Molecular aspects of lens cell differentiation. Science **156**: 338–346.

Piatigorsky J (1981): Lens differentiation in vertebrates. A review of cellular and molecular features. Differentiation **19**: 134–153.

Qiu X, Yang J, Liu T, Jiang Y, Le Q & Lu Y (2012): Efficient generation of lens progenitor cells from cataract patient-specific induced pluripotent stem cells. PLoS ONE **7**: e32612.

Schild G, Schauersberger J, Amon M, Abela-Formanek C & Kruger A (2005): Lens epithelial cell ongrowth: comparison of 6 types of hydrophilic intraocular lens models. J Cataract Refract Surg **31**: 2375–2378.

Tamiya S & Delamere NA (2005): Studies of tyrosine phosphorylation and Src family tyrosine kinases in the lens epithelium. Invest Ophthalmol Vis Sci **46**: 2076–2081.

Wormstone IM, Wang L & Liu CS (2009): Posterior capsule opacification. Exp Eye Res **88**: 257–269.

Yang J, Luo L, Liu X, Rosenblatt MI, Qu B, Liu Y & Liu Y (2010): Down regulation of the PEDF gene in human lens epithelium cells changed the expression of proteins vimentin and αB-crystallin. Mol Vis **16**: 105–112.

Yap AS, Crampton MS & Hardin J (2007): Making and breaking contacts: the cellular biology of cadherin regulation. Curr Opin Cell Biol **19**: 508–514.

Zelenka PS, Gao CY & Saravanamuthu SS (2009): Preparation and culture of rat lens epithelial explants for studying terminal differentiation. J Vis Exp **22**: 31. pii: 1519.

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