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Cell biology of posterior capsular opacification

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Abstract

Posterior capsular opacification (PCO), a major complication of modern cataract surgery, necessitates further surgical intervention in 10–50% of patients. PCO results from the growth and transdifferentiation of lens epithelial cells left on the anterior capsule at the time of cataract surgery. These cells proliferate to form monolayers on the capsular surfaces, and such monolayers continue to line the anterior capsule leaflet many years after surgery. Some cells, however, differentiate or undergo a transition to another cell type, and these processes greatly contribute to PCO. Equatorial differentiation of cells to fibre-like structures leads to Soemmerring's ring formation and peripheral thickening of the capsular bag. Closer to the rhexis, cell swelling can result in globular Elschnig's pearls, which may occlude the visual axis. Cells at the rhexis edge and those in the space around the optic appear to undergo epithelial–mesenchymal transition. The resulting cells are fibroblastic in morphology, express the smooth muscle isoform of actin and secrete extracellular matrix containing proteins not normally present in the lens.

Key words Actin, Capsule, Collagen, Epithelia, Proliferation, Transdifferentiation

In spite of continual refinements in surgical techniques and the development of new materials for intraocular lens production, posterior capsular opacification (PCO) remains as a major complication of modern cataract surgery.[1,2] In recent years it has become accepted that PCO is the result of the resilient growth of residual lens epithelial cells on the capsular bag.[1,3,4] Lens epithelial cells and equatorial cells remain attached to the anterior capsule at the time of the operation and these cells proliferate to re-populate the anterior capsule and grow across the posterior capsule. Further development can lead eventually to the formation of a peripheral Soemmerring's ring and/or capsular wrinkles and Elschnig's pearls which can occlude the visual axis. A secondary loss of visual acuity occurs in 10–50% of patients (including almost all young patients) in 3–5 years.[1,2]

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The appearance of cells both on the intraocular lens (IOL) and in the region of the central posterior capsule have been recorded in many clinical investigations (reviewed by Apple *et al.*[1]). Small cells (both round and fibroblastic) and giant cells, including macrophages, are commonly found on the anterior IOL in the immediate post-operative period.[5–7] In a recent study Shah and Spalton[8] used specular microscopy to follow the fate of these cells and showed that cell numbers peaked at between 1 and 3 months post-operatively and then normally declined significantly to very low numbers.

The early presence of cells on the posterior capsule is frequently observed by specular microscopy as the appearance of a cellular membrane progressing across the surface.[9] This stage corresponds to the presence of an initial monolayer of cells and appears to have few visual consequences.[1,10] It is rather the continued development into structures that scatter light which is responsible for loss of transparency. These developments include aggregation of cells into multilayered islets with regression of cells from some adjacent areas of the posterior capsule, the appearance of swollen cellular structures, the increased deposition of extracellular matrix and wrinkle formation in the capsule.

Normal lens epithelial cells are cuboid, express only the F-isoform of actin and produce extracellular matrix containing mainly collagen IV. However, cells with a fibroblastic morphology have been noted in clinical and experimental studies of PCO[1,11,12] and cells expressing the alpha-smooth-muscle isoform of actin (α-sma) and collagens I, III, V and VI, in addition to IV, have been detected by immunohistochemistry in the region around the IOL in human capsular bags.[13,14]

These features are characteristic of cells which have undergone a process of epithelial–mesenchymal transition (EMT). EMT is a well-known phenomenon which has been observed in a number of ocular tissues and tissue-cultured cells.[15–17] Post-translation cells have a myofibroblastic morphology and are marked by expressing α-sma as well as producing several forms of collagen. The process of EMT is driven by cytokines, of which transforming growth factor-beta (TGF-β) is the most important. TGF-β is a member of a cytokine family

involved in regulation of cell function including cell proliferation and differentiation (reviewed by Roberts and Sporn[18]) and extracellular matrix production. Both TGF-β and its mRNA have been found in ocular tissues close to the lens[19,20] and TGF-β is present in aqueous humour[21,22] and in cultured canine[23] and human capsular bags (I.M. Wormstone, personal communication).

A rat model has been used to investigate in detail the effect of growth factors, including TGF-β, on the whole lens and explanted capsule/lens epithelial cell preparations.[12,15,16,24] These studies have shown conclusively that in this model TGF-β induces the formation of wrinkles in the capsule and cataractous plaques containing fibroblastic, α-sma positive cells, surrounded by substantial layers of collagen, including collagens I and III. The changes observed indicate that the lens epithelial cells had undergone transition to a mesenchymal, myofibroblastic type.[15,16]

The changes associated with PCO have been investigated experimentally in a number of animal models[25,26] and with animal and human lens epithelial cells in tissue culture.[27–29] In order to gain a better understanding of the morphology and disposition of viable cells in *ex vivo* capsular bags with IOLs from donor eyes, the Norwich Eye Research group has recently undertaken an investigation in collaboration with the Netherlands Ophthalmic Institute (Marcantonio, Vrensen *et al.*, submitted). The bags obtained for the study had been *in situ* for periods from 4 months to 13 years. Combinations of dark-field, phase and polarising microscopy, epifluorescence immunocytochemistry and electron microscopy (SEM and TEM) were employed to study whole mounts and sections.

The most remarkable finding in relation to the investigation of cell structure in *ex vivo* capsular bags from patients is the persistence and resilience of lens epithelial cells in the region enclosed by the two capsular leaflets. Even as late as 13 years after IOL implantation this region of the bag was found to be covered with lens cells which were largely indistinguishable from those of a native epithelium in overall morphology and cytoskeletal structure. Cells attached to the anterior capsule flap had a cobblestone appearance by phase microscopy (Fig. 1a, b) and were cuboid in section like native epithelial cells. Their F-actin cytoskeleton (Fig. 1c) and vimentin, tubulin and spectrin distributions and nuclear size and shape were also similar to those of native lens epithelial cells. The cells growing on the posterior capsule were more flattened and showed more inter- and intracellular spaces, but otherwise contained the same organelles as the anterior capsule cells. In many

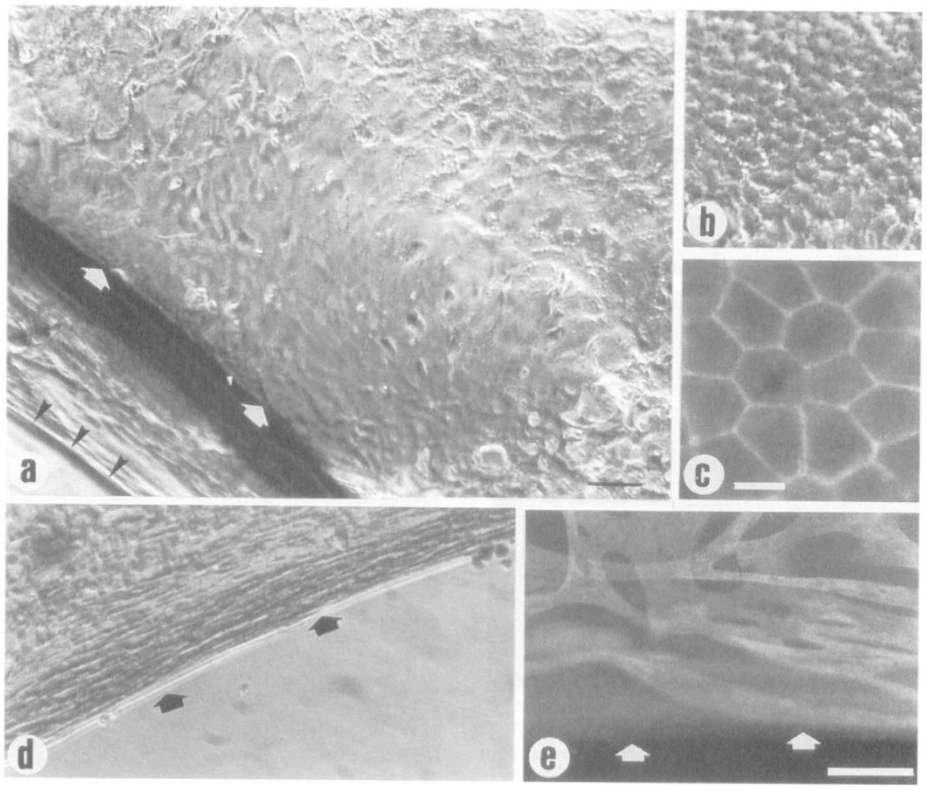

Fig. 1. *(a) Phase micrograph of an unfixed capsular bag from the posterior aspect. The anterior rhexis (arrowheads) is visible through the IOL (arrows) and the outlines of the lens epithelial cells covering the anterior capsule can be seen. Scale bar represents 75 μm. (b) Phase micrograph of the cobblestone appearance of fixed lens epithelial cells on the anterior capsule of a capsular bag. Magnification as in (a). (c) Epifluorescence micrograph of the F-actin cytoskeleton of lens epithelial cells on the anterior capsule, where the cortical F-actin layer outlines hexagonal cell borders. (F-actin stained with Texas red-conjugated phalloidin.) Scale bar represents 25 μm. (d) Phase micrograph of the rhexis edge in a fixed capsular bag, showing the bands of elongated cells running round the rhexis (arrows) (cf. Fig. 2b). Magnification as in (a). (e) Epifluorescence micrograph of cells at the rhexis edge (arrows) showing positive staining for alpha smooth muscle actin. (Cells stained with anti-α-sma (clone 1A4), detected with FITC-conjugated anti-mouse.) Scale bar represents 100 μm.*

places the apical surfaces of the two cell layers were in very close apposition, forming a cellular adhesion zone which resulted in the presence of a closed system between this zone and the equator of the bag. Within this system the cells at the equator appeared to behave like the cells at the native lens bow, in that elongation of cells and loss of organelles occurred, leading to the presence, in some cases, of a Soemmerring's ring. Close to the equator these elongated cells frequently showed regular organisation similar to lens fibre formation. The part of the bag enclosed by the double capsule leaflets, between the IOL edge and the equator, is therefore extremely lens-like in its cellular structure, apart from the presence of epithelial-like cells on the posterior as well as the anterior capsule.

Close to the rhexis, however, in the space around the edge of the IOL, globular structures were more common and it is probable that it is these structures which can protrude from the double leaflet into the central area, above or beneath the IOL, forming Elschnig's pearls.

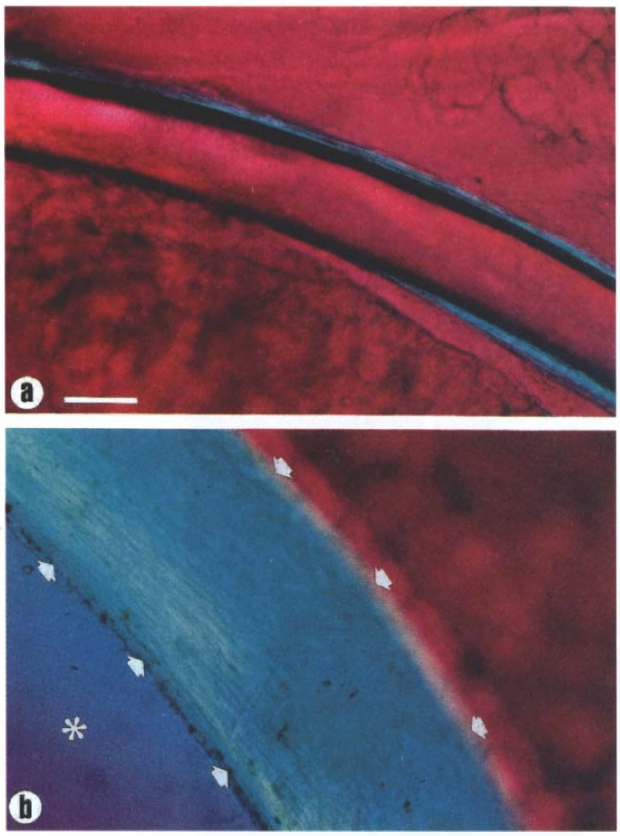

Fig. 2. *Polarising microscopy patterns obtained from unfixed human capsular bags. The preparation was viewed through a polariser and analyser set at 90° and a first-order red plate inserted at 45° to both axes. Molecules or cells lined up along the first-order red plate give the blue addition colour. In the red areas there is no significant degree of cellular order in the system. (a) The blue addition colour is derived from elongated cells that are aligned with their axes parallel to the IOL haptic. Scale bar represents 500 μm. (b) The blue region between the arrows arises from strands of elongated cells located on both the outer and inner faces of the anterior capsule and aligned around the rhexis (cf. Fig. 1d). The plastic of the IOL optic also results in a small degree of polarisation (asterisk). Magnification as in (a).*

At the rhexis edge itself cells migrate onto the anterior surface of the anterior capsule to form a supracapsular monolayer, as has been observed by Champion *et al.*[30] and Kappelhof *et al.*[31] Our study, in addition, showed that many of these cells, and those present on the inner face of the rhexis edge, did not retain their epithelial-like morphology, but elongated and formed bands running round the rhexis border (Fig. 1d). The alignment of cells in this region resulted in a blue addition colour when the specimens were viewed by polarising microscopy (Fig. 2b). Investigation of the cytoskeleton revealed that the cells were positive for α-sma (Fig. 1e).

In the open system around the IOL the confluent monolayers of epithelial cells were absent, and were replaced by three-dimensional, multilayered stands and aggregates of cells which had lost their direct attachment to the capsule (Fig. 3a). The cells showed two distinct morphologies (Fig. 3b), with very elongated cells containing long nuclei in the strands and more rounded cells with ovoid nuclei in the aggregates. A few small patches of capsule-attached epithelial cells were found in some specimens. All the cells in this region of the bags were positive for α-sma expression (Fig. 4a). Fig. 4a also shows that the wrinkles which were present in the posterior capsule did not contain a viable cell population and were in a different plane of focus from the cell

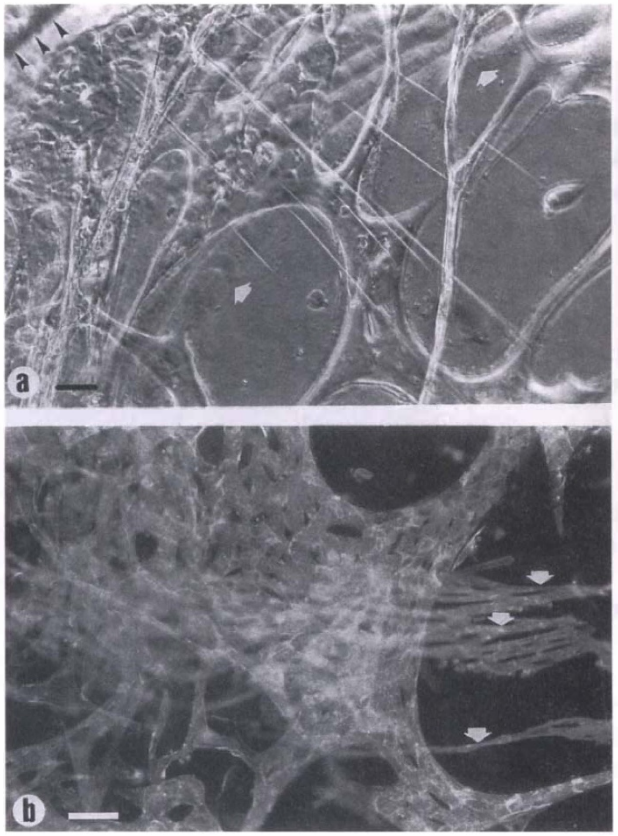

Fig. 3. *(a) Phase micrograph of unfixed multilayered strands and islands of cells on the posterior capsule beneath the IOL. The rhexis edge (arrows) and IOL edge (arrowheads) are out of focus. Scale bar represents 75 μm. (b) Epifluorescence micrograph of the F-actin cytoskeleton of cells from the same region. Islands of cells of intermediate morphology and strands of elongated cells (arrows) occupy the space in different planes of focus. Scale bar represents 75 μm.*

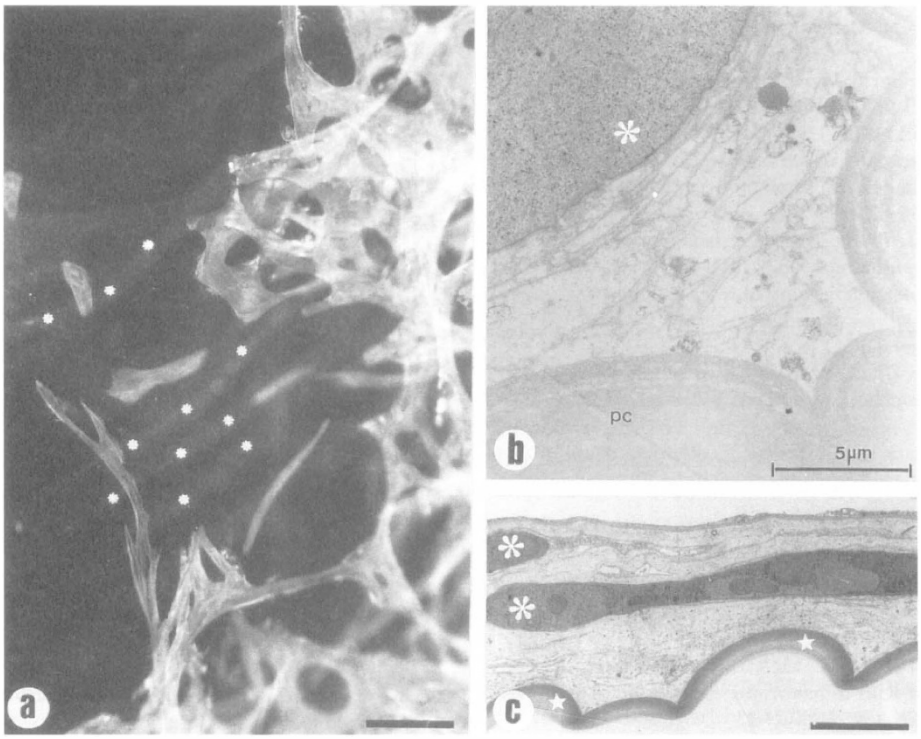

Fig. 4. *(a) Epifluorescence micrograph of multilayers of α-sma positive cells on the posterior capsule, overlying wrinkles (small asterisks) which are free of cells. Scale bar represents 100 μm. (b) Transmission electron micrograph (TEM) shows the edge of a group of cells (asterisk) separated from the posterior capsule (PC) by a region containing loosely ordered fibrils and basement-membrane-like material. Scale bar represents 5 μm. (c) TEM of strands of electron-dense cells (asterisks) surrounded by a substantial layer of extracellular matrix containing some cell debris, above the posterior capsule (stars) which is wrinkled and is not in intimate contact with viable cells. Scale bar represents 20 μm.*

strands. TEM shows that the groups of viable cells were surrounded by extracellular matrix which was much less compact than the capsule (Fig. 4b) and which filled the wrinkles. This extracellular matrix appeared to contain some cell debris and in some specimens very thick layers were present (Fig. 4c).

It is clear that the cells found in the 'open' part of the bags in this study demonstrated the main markers of fibroblastic/myofibroblastic cells, in that they expressed α-sma and were surrounded by layers of new extracellular matrix. Since it has been demonstrated both clinically and in model systems that the initial cell population is epithelial,[1,4,28,32] it appears that *in vivo* most cells in the area around the IOL undergo EMT, and that it is this process which is responsible for the thickening and wrinkling of the posterior capsule.

The continued presence of viable cells within the capsular bags means that further changes to structure and transparency could follow any disturbance of the system. Rapid proliferation of cells following Nd-YAG laser capsulotomy has been recorded clinically,[33] especially in patients with retinal pathology; moreover, closure of the aperture in the posterior capsule has been observed in a human model system.[34] One specimen of post-laser-capsulotomy has occurred in our own recent study. This bag was removed 3 weeks after laser treatment, and it was found that the posterior capsule had split across into the region under the anterior capsule flap. There was clear evidence that epithelial-like cells were again growing over the surface of the remaining capsule.

In order to study the PCO phenomenon in the laboratory we have developed a human model[32] in which a sham cataract operation is used to create capsular bags from donor lenses. The bags (with or without an implanted IOL) are cultured in defined medium without added serum or growth factors[35] and can be maintained for many months, during which time cell growth and development can be monitored. Many of the features of *in vivo* bags have been observed in the cultured bags, including the development of epithelial cell sheets on both anterior and posterior capsule, wrinkle formation and cell aggregation into multilayered islets. Incubation with radiolabelled amino acids and subsequent analysis have shown that the cells continue to produce proteins after several months in culture and that the bags contain measurable amounts of cytokines such as basic FGF and HGF[36] and TGF-β (I.M. Wormstone, personal communication). This model will be used for further study of PCO development and prevention[37] in both laboratory-created and *ex vivo* human capsular bags.

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