An In Vitro Human Lens Capsular Bag Model Adopting a Graded Culture Regime to Assess Putative Impact of IOLs on PCO Formation

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Purpose. To develop a culture regime for the in vitro human lens capsular bag model that better reflects clinical events following cataract surgery and to use this refined model to evaluate the putative impact of IOLs on PCO formation.

Methods. Capsulorhexis and lens extraction were performed on human donor eyes to generate capsular bags attached to the ciliary body by the zonules. Preparations were secured by pinning the ciliary body to a silicone ring and maintaining in 6 mL serum-free EMEM for 28 days or in a graded culture system (days 1–3, 5% human serum and 10 ng/mL TGFβ2; days 4–7, 2% human serum and 1 ng/mL TGFβ2; days 8–14, 1% human serum and 0.1 ng/mL TGFβ2; days 15–28, serum-free EMEM), which better mimics clinical changes. Preparations were monitored with phase-contrast and modified-dark-field microscopy. Cell coverage and light scatter were quantified using image analysis software. The transdifferentiation marker, α-SMA and matrix component, fibronectin were assessed by immunocytochemistry. To assess IOLs in the model, Alcon Acrysof or Hoya Vivinex IOLs were implanted in match-paired capsular bags.

Results. Match-paired experiments showed that graded culture enhanced growth, facilitated matrix contraction, increased transdifferentiation, and promoted matrix deposition relative to serum-free culture. The graded culture protocol was applied to match-paired bags implanted with a Hoya Vivinex or an Alcon Acrysof IOL. The Vivinex demonstrated a lag in growth across the posterior capsule. However, by day 28, coverage was similar, but light-scatter was greater with Acrysof implanted. Cell growth on the Acrysof IOL anterior surface was significantly greater than Vivinex.

Conclusions. The graded culture human capsular bag model serves as an excellent system to evaluate and develop intraocular lenses. The Hoya Vivinex IOL showed an overall better level of performance against postsurgical wound healing and PCO than the Alcon Acrysof using this model.

Keywords: human, lens, cataract, posterior capsule opacification, model, IOL

In the majority of cases, cataract is a consequence of the aging of the lens and is the major priority in the global initiative to eliminate avoidable blindness by the year 2020.[1] At present, the only means of treating cataract is by surgical intervention, and this initially restores high visual quality. Unfortunately, posterior capsule opacification (PCO), the most common complication of cataract surgery, develops in millions of patients worldwide to such an extent that a secondary loss of vision occurs.[2]

The intraocular lens (IOL) is an important component within cataract surgery; in addition to restoring refractive power, it can also affect the progression of PCO.[2–7] At present it is widely believed that an IOL with a square-edge profile and complete contact between the IOL and the capsulorhexis offers the best PCO prevention. Preclinical testing of IOLs most commonly employs the rabbit due to the similar dimensions of the human and rabbit lenses. While the rabbit provides an in vivo test system, postsurgical responses are severe, the experimental period is usually months, and the factors driving PCO will differ from human.[7,8] Evaluation of IOLs can also be performed using human capsular bag models. These systems have served as a valuable tool to understand the biologic processes governing PCO and have been used to evaluate clinical products including IOLs.[9] To enhance the utility of the capsular bag model for evaluation and development of IOLs, the model has evolved with time and incremental improvements have been made. The first iteration secured (anterior face up) the capsular bag containing an IOL to a dish using entomological pins at the equator.[10] While this was useful for assessing the use of the IOL as a drug delivery system,[11] it failed to allow interaction between the IOL and capsule as seen in patients. To improve interaction between the IOL and capsule a modification was introduced, such that the bag was secured to the dish with the anterior capsule down.[12] When the periphery was secured, this led to good interaction between the optic edge and the capsule. Further modifications were made that replaced fetal calf serum with human serum and the application of TGFβ was also introduced to further mimic biologic events associated with PCO in humans.[12] Cleary et al.[13] adapted the model to

maintain integrity of the capsular bag. To achieve this, the ciliary body was retained in addition to the capsular bag generated from simulated cataract surgery. The ciliary body is secured to a silicone ring, which allows the bag containing an IOL to be suspended by the zonules over the lumen of the ring. This allows a natural interaction between the IOL and capsular bag to occur. Using this system, IOLs have been previously assessed,[3] but conditions involved either serum-free or human serum supplemented medium as the maintenance medium for the experimental duration.[3] However, the previous models did not reflect the clinical observations for patients, where the elevation of proteins in the aqueous humor following surgery is transient, such that levels peak in the first week then decline to baseline.[14] With this in mind, our approach in this current study was to employ culture conditions that better reflect this general pattern of response and validate that such conditions could mimic features previously reported in postmortem capsular bag samples.[15] This was achieved by establishing a culture protocol based on clinical data[14] that has shown increased levels of protein in the eye (detected by flare measurements) following surgery that peak within the first week and then decline to baseline levels. Similarly, TGFβ levels are believed to rise following surgery and we assume this elevation will also be transient.[2,16] We describe the conditions that employ transient elevation of serum and TGFβ in this study as a graded culture system. Using graded culture conditions, it is possible to use this modified and improved model to assess the relative "barrier" function of IOLs, progression of cell cover within the visual axis (on the posterior capsule and IOL), light scatter, and cell behavior following reduction/removal of exogenously applied serum/TGFβ All these features contribute to PCO and thus can help better predict the potential clinical performance of IOLs.

Methods

Capsular Bag Preparation

The model previously described by Liu et al.,[10] developed by Cleary et al.[13] and further refined by Eldred et al.[3] was employed with additional refinements. Donor human eye globes were obtained with UK National Research Ethics Committee approval (REC 04/Q0102/57) and used in accordance with the tenets of the Declaration of Helsinki. A small capsulorhexis was introduced on the anterior surface of the lens capsule, and the central fibrous mass was removed via hydrodissection, revealing the capsular bag. In some cases, an IOL (Hoya Vivinex or Alcon Acrysof) was implanted. The capsular bag (with or without an IOL implanted) and a ring of ciliary tissue secured to a silicone ring was transferred to a tissue culture dish. This arrangement allows capsular bags to suspend from the zonules, and were maintained in defined culture conditions over 28 days. To better reflect the clinical changes to the lens environment post-surgery,[14,16] the defined culture system was initiated with elevated protein (serum and TGFβ) levels that were gradually reduced to a serum-free environment, as follows: days 1 to 3, 5% human serum and 10 ng/mL TGFβ2; days 4 to 7, 2% human serum and 1 ng/mL TGFβ2; days 8 to 14, 1% human serum and 0.1 ng/mL TGFβ2; days 15 to 28, serum-free EMEM. Two series of match-paired experiments were performed in the current study. The first series was to establish the graded-culture system, such that match-paired experiments were performed using two lenses from the same donor, where one preparation was treated with graded culture while the partner capsular bag was maintained in untreated serum-free control medium. The second match-paired series was used to evaluate two IOLs using the graded-culture system, the match-paired experiments were carried out using two lenses from the same donor both maintained in the graded culture system, where one capsular bag was implanted with an Alcon Acrysof IOL and its partner was implanted with a Hoya Vivinex IOL. Cell coverage of the previously cell-free posterior capsule, and additional surfaces such as the IOL, were captured using low-power phase microscopy. The level of cell cover on the posterior capsule (PC), within the visual axis, was quantified from micrographs using ImageJ analysis software (http://imagej.nih.gov/ij/; provided in the public domain by the National Institutes of Health, Bethesda, MD, USA). To achieve this, low-power dark-field images of capsular bags, with IOLs removed, were captured at endpoint and analyzed, such that the central visual axis (outlined by the capsulorhexis) was defined and its area quantified. The areas within this region of interest covered by cells was manually defined and the area of all cell covered regions quantified. The area of cell covered regions was then expressed as a percentage of the central visual axis area.

Quantification of Light Scatter

Modifications to the posterior capsule cause light scatter and are significant contributors to visual disturbance associated with PCO. To establish a measure of light scatter in the central PC, Image Pro Premier software (Media Cybernetics, Warrendale, PA, USA) was employed using a published method adapted from Wormstone et al.[17] In the present study, modified dark-field images of the central posterior capsule, obtained following IOL removal, were placed through a single pass of a Laplacian filter, which identifies light scattering regions. These regions appear bright, and thus have a high intensity per pixel relative to nonscattering regions. The standard deviation of the pixel intensities within the rhexis region was determined. Capsular bags without cells present on the PC were used to establish background levels, which was subtracted from test values.

Immunocytochemistry

Transdifferentiation of epithelial cells to myofibroblasts and deposition of the matrix component fibronectin are clinical features of PCO. We, therefore, employed immunocytochemistry to visualize and quantify expression of the myofibroblast marker, α-SMA, and fibronectin. All reagents were from Sigma-Aldrich (Poole, Dorset, UK) unless otherwise stated. Washes were for 3 × 15 minutes in PBS. IOLs were removed from capsular bag preparations then fixed for 30 minutes in 4% formaldehyde in PBS and permeabilized in PBS containing 0.5% Triton-X100, also for 30 minutes. Nonspecific sites were blocked with normal goat serum (1:50 in 1% BSA/PBS). Anti-alpha smooth muscle actin or anti-fibronectin was diluted 1:100 in 1% BSA/PBS and applied for 60 minutes at 35°C, followed by washing. Alpha smooth muscle actin and fibronectin presence was visualized with AlexaFluor 488–conjugated secondary antibodies (Molecular Probes, Leiden, The Netherlands). The stained preparations were again washed extensively, floated onto microscope slides, and mounted in mounting medium (Hydromount; National Diagnostics, Hull, UK). Images were viewed with a epifluorescence microscope and software (Axiovision; Carl Zeiss AG, Oberkochen, Germany).

Measuring Cell Cover on the Posterior and Anterior Surfaces of the IOL

At endpoint, IOLs were carefully removed from the capsular bag prior to fixation by creating radial incisions close to the

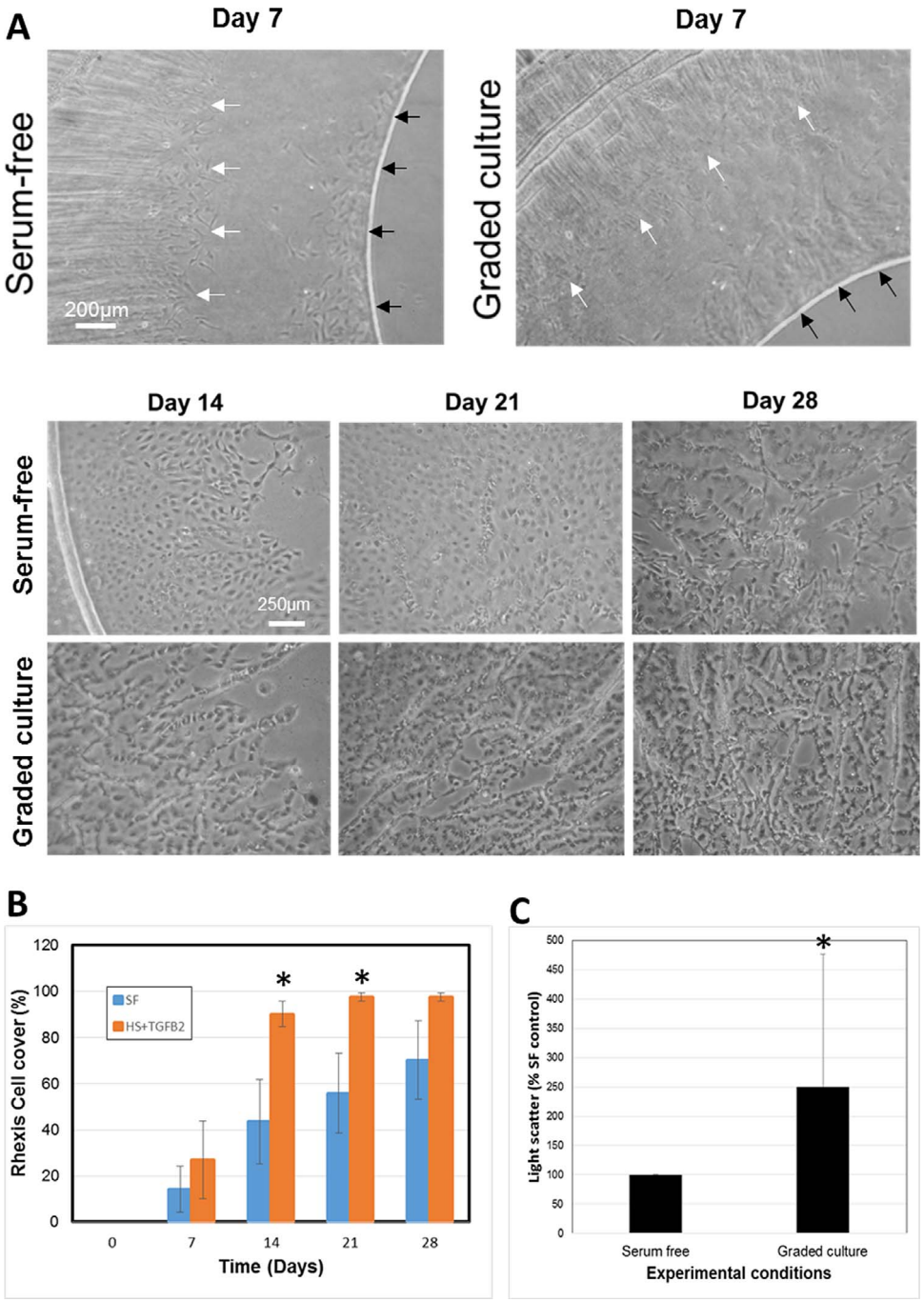


Figure 1. Assessment of growth characteristics and matrix modification in capsular bags maintained under graded culture (days 1–3, 5% human serum and 10 ng/mL TGFβ2; days 4–7, 2% human serum and 1 ng/mL TGFβ2; days 8–14, 1% human serum and 0.1 ng/mL TGFβ2; days 15–28 serum-free) or serum-free (SF) culture conditions. (**A**) Progressive movement of cells (leading edge indicated by *white arrows*), initially on the peripheral posterior capsule (day 7), then beyond the rhexis margin (*black arrows*) onto the central posterior capsule (days 14, 21 and 28). Note that wrinkling of the posterior capsule is associated with movement in graded cultures, whereas serum-free cultures exhibit matrix wrinkling once movement has been arrested. (**B**) Quantitative analysis showing cell coverage of the central posterior capsule (within the rhexis margin) in serum-free and graded culture conditions. Data are presented as mean ± SD ($n = 5$). *Indicates a significant difference between groups (Student's *t*-test; $P \leq 0.05$). (**C**) Pooled data showing light scatter of the central PC in capsular bags that had been cultured for 28 days in serum-free or graded culture conditions. Data are presented as mean ± SD ($n = 5$). *Indicates a significant difference between groups (Student's *t*-test; $P \leq 0.05$).

haptic arms. This allowed the haptics to be held by fine forceps and the IOL to be carefully removed from the capsular bag with minimal disruption to cell populations. The IOL was then immediately placed in fixative (4% formaldehyde) for 30 minutes followed by washing in PBS. The IOL was then placed in Coomassie Brilliant Blue for 1 hour. Following this period, the IOLs were washed multiple times in PBS to remove excess dye. The stained IOLs were imaged using bright-field illumination and a charge-coupled device (CCD) camera (UVP, Cambridge, UK) with Synoptics software (Synoptics, Cambridge, UK). The amount of cell coverage was determined using ImageJ 1.45s image analysis software.

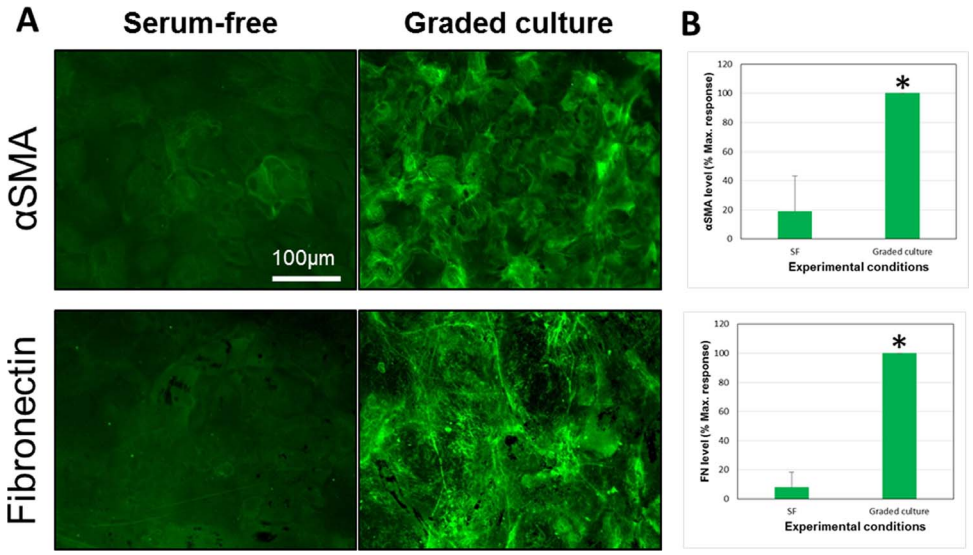


FIGURE 2. (**A**) Representative epifluorescence micrographs showing the distribution of the myofibroblast marker, α-smooth muscle actin and the matrix component, fibronectin associated with cells growing on the central posterior capsule of human lens capsular bag preparations maintained in SF or graded culture conditions. Images were captured at endpoint (day 28). (**B**) Pooled data showing quantitative αSMA and fibronectin expression in association with cells growing on the central PC of capsular bags maintained in serum-free or graded culture conditions. Data are presented as mean ± SD ($n = 5$). *Indicates a significant difference between groups (Student's *t*-test; $P \leq 0.05$).

RESULTS

Establishing a Graded Culture Capsular Bag Model

Through application of the graded culture protocol, it was predicted that the presence of serum would stimulate growth and TGFβ would promote EMT, matrix deposition, and matrix contraction (capsule wrinkling), which are all clinical features of PCO.[2] Match-paired experiments were established from five donors (range: 42–82 years; mean age: 60.2 ± 15.9 years; 1 male and 4 female) with one donor used for each match-paired experiment. It was evident within the first week of culture that cells had recolonized denuded regions of the anterior capsule and had started to migrate across the posterior capsule beneath the overlying anterior capsule in graded culture and serum-free control conditions (Fig. 1A). At day 14, cells had progressed beyond the rhexis margin and entered the central visual axis (Fig. 1A). Cells continued to cover the posterior capsule for the remaining culture period, but the rate of progression was significantly greater with graded culture conditions (Figs. 1A, 1B). It was also evident in the graded culture preparations that matrix contraction occurred as cells covered the posterior capsule, whereas serum-free cultures migrated as a sheet of cells and only induced matrix contraction when movement had ceased (Fig. 1A). Overall, match-paired comparison revealed a significant difference in light scatter between the graded culture and serum-free control treatments (Fig. 1C). At the endpoint of 28 days, preparations were fixed and assessed for the matrix component, fibronectin, and the myofibroblast marker, α-smooth muscle actin. It was found that both fibronectin and α-smooth muscle actin were significantly increased with graded culture relative to serum-free control (Fig. 2). The graded culture regime applied to capsular bags; therefore, enhanced growth, promoted matrix contraction, increased EMT markers, and promoted matrix deposition. These features reflect previously reported post-mortem analysis[15] and thus suggest the capsular bag model maintained in graded culture conditions is a suitable model to evaluate the ability of different IOLs to moderate PCO following implantation during cataract surgery. In the present study, we compared Alcon Acrysof and Hoya Vivinex IOLs using this test system.

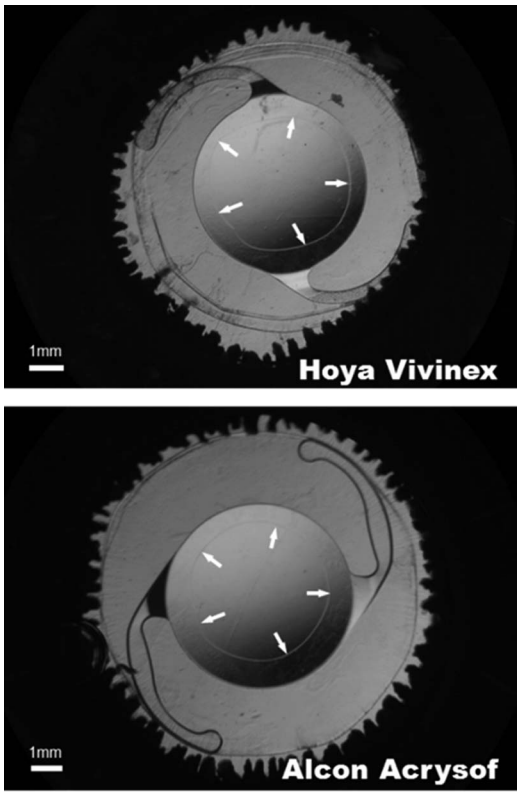


FIGURE 3. Representative modified dark-field images of match-paired capsular bags implanted with a Hoya Vivinex or an Alcon Acrysof IOL captured on the day of simulated surgery. In both cases the capsulorhexis (*arrowed*) is fully seated on the IOL optic.

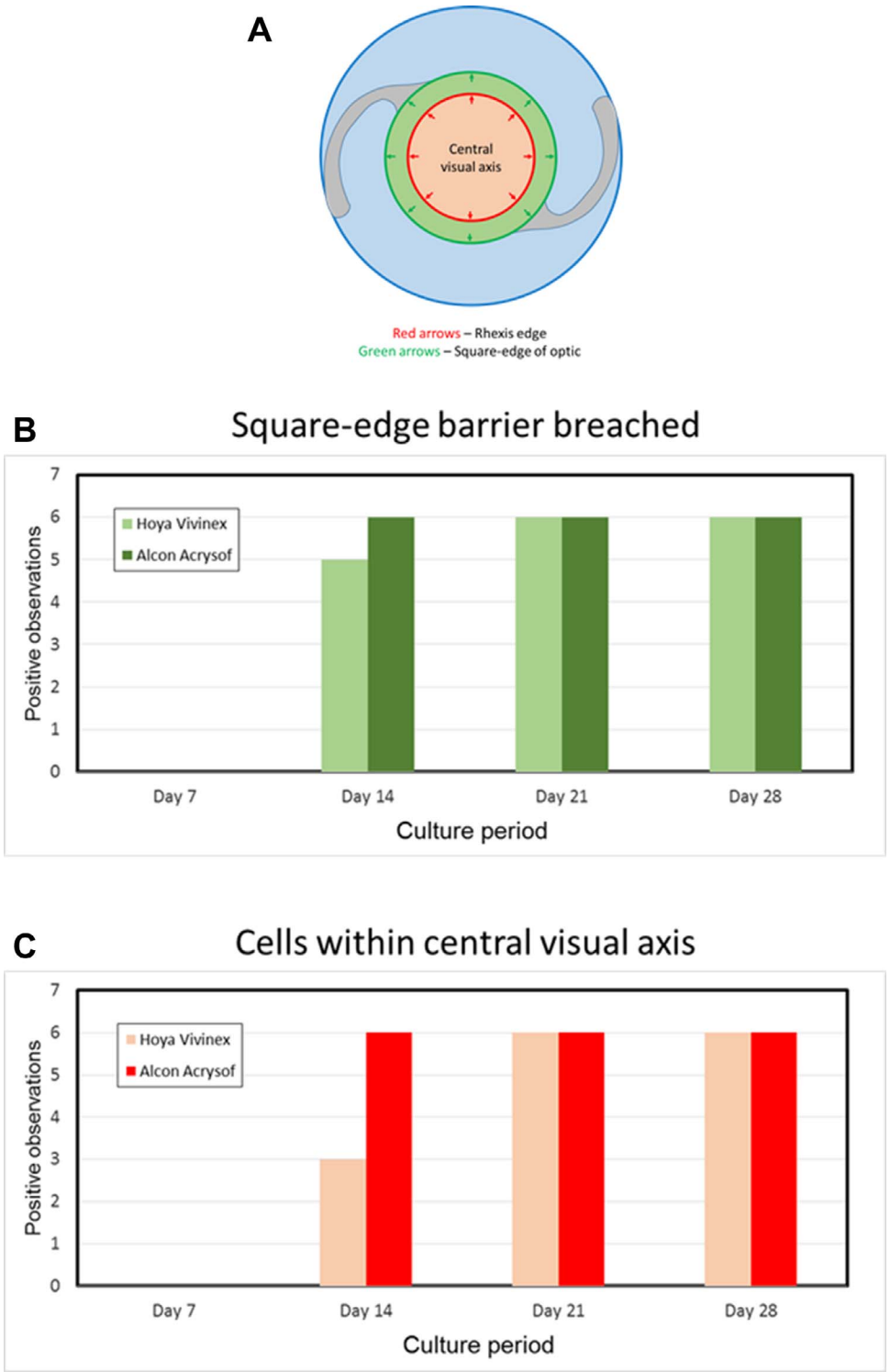


Figure 4. Observation of cell growth progression on the posterior capsule in capsular bag preparations implanted with a Hoya Vivinex or Alcon Acrysof IOL. The data represent observations from six match-paired capsular bag preparations. A schematic shows the peripheral edge region (*green*) and central visual axis region (*red*) of the IOL (**A**). The number of IOLs with cell presence on the peripheral edge (**B**) and the central visual axis (**C**) were quantified, in which both analysis showed that Hoya Vivinex IOLs have reduced presence of cells at day 14 compared to Alcon Acrysof IOLs.

IOL Evaluation Using the Graded Culture Capsular Bag Model

Twelve eyes from six donors (range 50–84 years; mean age 70 ± 10.3 years; 2 male and 4 female) with one donor used for each match-paired experiment in this study. There was no discernible difference in the ease of implantation between the Hoya Vivinex and Alcon Acrysof IOLs. Similar sized capsulorhexes (4.5–5 mm diameters) were generated and in all cases the capsulorhexis was completely mounted on the anterior IOL optic face (Fig. 3).

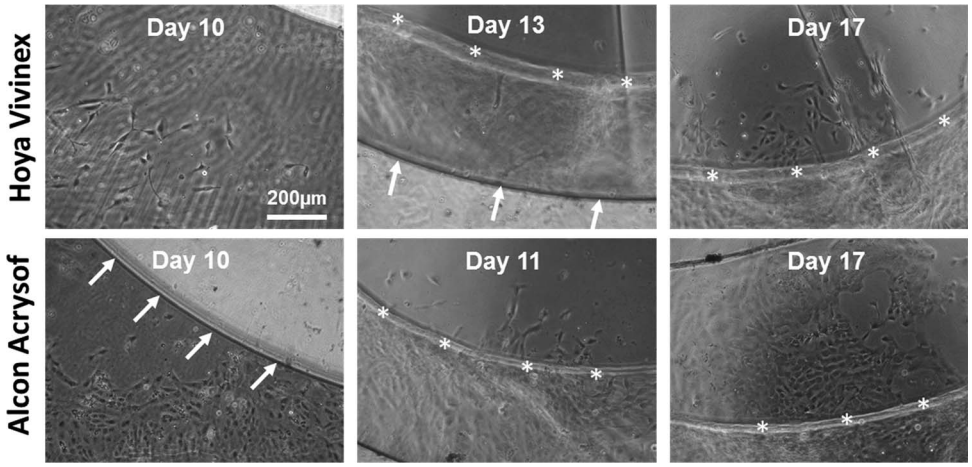


FIGURE 5. Representative phase-contrast micrographs showing progressive cell growth on the posterior capsule of match-paired capsular bags implanted with a Hoya Vivinex or Alcon Acrysof IOL. The capsulorhexis edge is highlighted by white asterisks. And the IOL optic perimeter is highlighted by *white arrows*.

Residual populations of lens epithelial cells on the anterior lens epithelium following simulated cataract surgery was comparable between each capsular bag preparation from a single donor (data not shown).

Cell Growth on the Posterior Capsule

Within the first few days following simulated surgery and IOL implantation, recolonization of denuded regions of the anterior capsule could be seen with either IOL present. Within the first week of culture no cells were observed on the posterior capsule underneath the IOL optic (i.e., the IOL square-edge barrier had not been breached; Fig. 4). As time progressed cells could be seen growing on the peripheral posterior capsule (beneath the anterior capsule; Fig. 5). In general, the rate of this coverage was to be slower with the Hoya Vivinex IOLs implanted compared to preparations implanted with an Alcon Acrysof IOL. This is illustrated by Figure 5, which shows growth of cells on the central posterior capsule (beyond the rhexis) at day 11 with an Acrysof IOL implanted, whereas the Hoya Vivinex implanted match-paired preparation presented cells past the IOL edge, but had not progressed beyond the rhexis edge at day 13 (Fig. 5). Following 2 weeks of culture, a number of preparations exhibited some cell growth on the posterior capsule beneath the IOL demonstrated that the IOL square-edge barrier had been breached (five out of six Vivinex versus six out of six Acrysof; Fig. 4). This difference was more pronounced when the central posterior capsule (PC), within the rhexis margin (which directly correlates to the central visual axis), was assessed. Three out of six Hoya Vivinex IOLs implanted preparations showed cells detectable within this region, while 6 out of 6 Alcon Acrysof IOLs implanted preparations had cells within the central visual axis (Figs. 4, 5). As the culture period was extended, cells continued to populate the posterior capsule as expected. At the endpoint of 28 days, all preparations exhibited some cell growth within the rhexis margin (Figs. 4, 6). Cell coverage within the rhexis margin (central visual axis) was not significantly different, such that the level of cover was quantified at 34.4% ± 25.4% and 37.4% ± 27.7% for Hoya Vivinex IOLs and Alcon Acrysof IOLs respectively (Fig. 6B). It should also be noted that matrix contraction on the central PC (central visual axis) was observed in most cases when cell growth in this region was seen. However, the degree of light scatter detectable in the central visual axis was significantly greater with Alcon Acrysof IOLs implanted relative to the Hoya Vivinex IOLs (Fig. 6C). While the degree of light scatter varied from donor to donor, it should be noted that detected light scatter was consistently lower with Hoya Vivinex IOLs in all six match-paired experiments with statistical significance measured at $P \leq 0.05$.

Cell Coverage on the IOL Anterior Surface

On the anterior face of the IOL, significantly more cell coverage was observed with Alcon Acrysof IOL implanted capsular bags than preparations implanted with a Hoya Vivinex IOL. While the degree of outgrowth onto the IOLs varied from donor to donor, the pattern of response was consistent in all six of the match-paired experiments (Fig. 7).

Within the first week of culture, in two out of six cases, cells were seen attached to the IOL, growing from the rhexis edge on to the Alcon Acrysof IOL anterior surface (Fig. 7A). No growth onto the IOL was observed at this stage when a Hoya Vivinex IOL was implanted. By day 14, four out of six Hoya Vivinex IOLs had observable cell outgrowth compared to five out of six Alcon Acrysof IOLs (Fig. 7A). This pattern was maintained until the final week of culture and at endpoint all 12 IOLs implanted exhibited some degree of coverage within the rhexis margin (Fig. 7B). The amount of cell coverage was significantly greater on Alcon Acrysof IOLs than their Hoya Vivinex counterparts (Figs. 7B, 7C). The reduced cell outgrowth on the Hoya Vivinex IOL anterior surface implies better optical performance due to a clearer light path through the IOL, which reduces cell-induced light scattering or glare.[18]

DISCUSSION

Refinement of the Capsular Bag Model for IOL Evaluation

It was clear from match-paired experiments that our graded culture conditions reflected clinical events associated with PCO, such that cell growth (proliferation/migration), matrix deposition, matrix contraction, and EMT were significantly enhanced relative to serum-free controls. Adopting this strategy reflects the general pattern of the postsurgical environment, such that there is a transient increase in putative exogenous stimuli followed by extended periods when the supply of exogenous stimuli is minimal. At this stage, progression is driven by lens cells and their interaction with

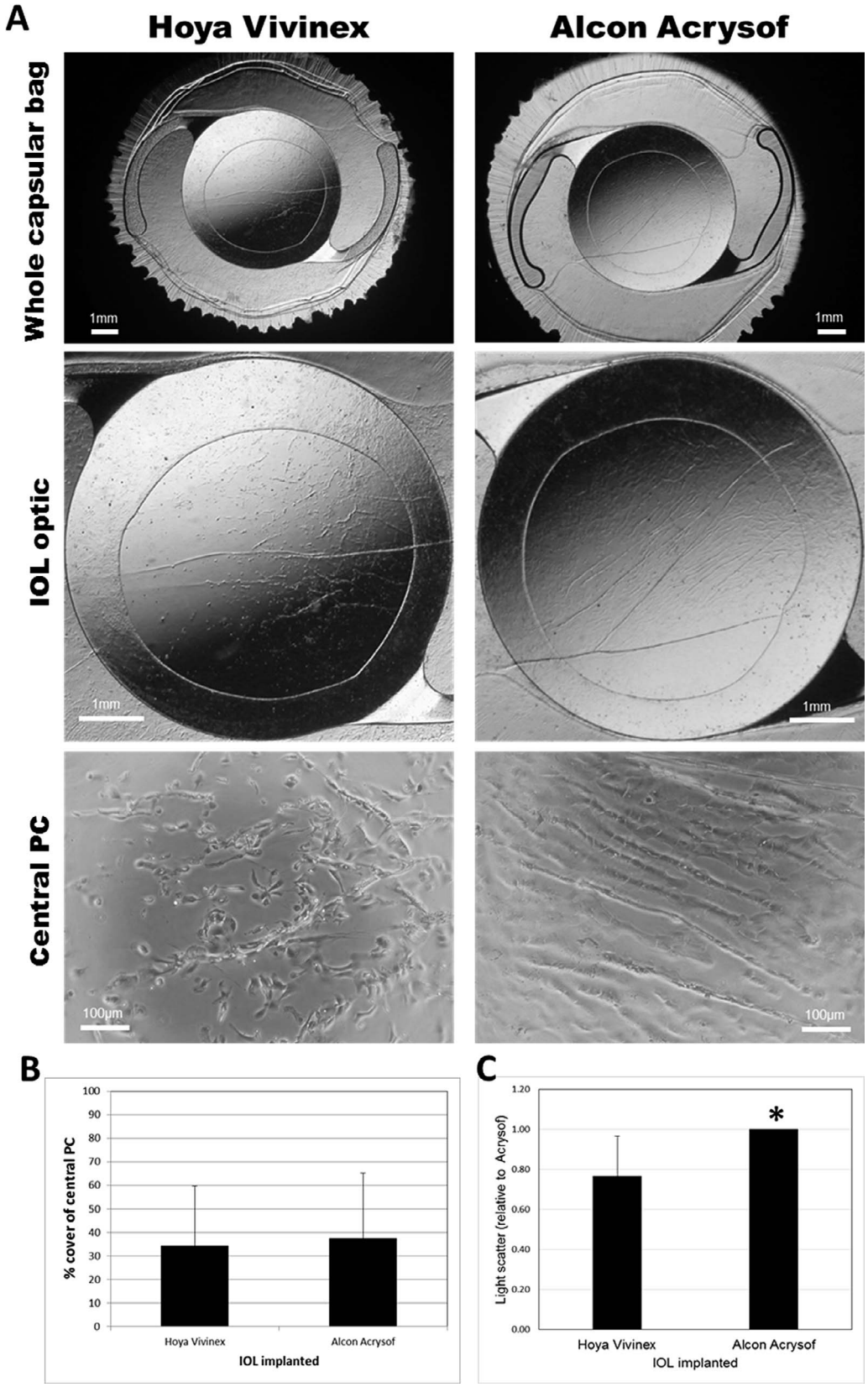
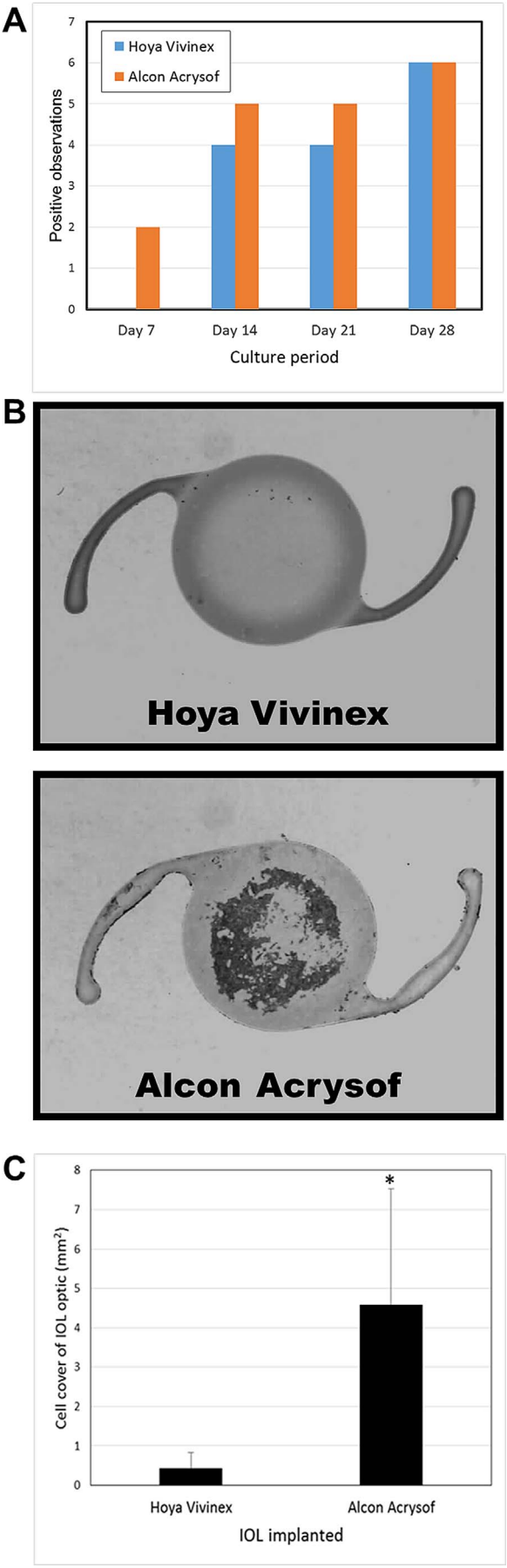


FIGURE 6. (**A**) Representative modified dark-field and phase-contrast images of capsular bags, captured prior to fixation, at the 28-day endpoint. (**B**) Pooled data showing coverage of the central PC by LECs, at the end of 28 days, in the presence of Hoya Vivinex or an Alcon Acrysof IOL. Data are presented as mean ± SD ($n = 6$). No significant difference was observed between groups (Student's *t*-test; $P \leq 0.05$). (**C**) Pooled data showing light scatter of the central PC in capsular bags that had been cultured for 28 days in the presence of Hoya Vivinex or an Alcon Acrysof IOL. Data are presented as mean ± SD ($n = 6$). *Indicates a significant difference between groups (Student's *t*-test; $P \leq 0.05$).



the lens capsule. It is known that a number of growth factors, such as FGF, HGF and VEGF can promote wound healing leading to PCO following cataract surgery.[2,9] Many of these factors become elevated in the eye following a breakdown of the blood aqueous barrier and thus addition of serum to our cultures mimics this process. TGFβ2 is the major TGFβ isoform within the eye and following surgery, activation will be increased in addition to greater production by ocular tissues.[16] As the increase in TGFβ2 within the eye is fundamentally a local event, TGFβ2 was added in addition to serum, so that both postsurgical changes could be modelled. The levels of serum and TGFβ used in the current model will represent a fairly extreme postsurgical response, but this affords the opportunity to provide a greater challenge to IOLs in relation to anti-PCO performance. Using our graded culture system, the initial drive from added serum allows the barrier function of the IOL to be tested. In addition, we can assess cell progression, light scatter and cell behavior as PCO is forming over time, rather than an endpoint measurement seen in rabbit studies and human clinical data. In the first instance, we elected to compare IOLs used in a large number of patients, which are hydrophobic acrylic lenses sold by market leaders: the Hoya Vivinex and the Alcon Acrysof. Both designs incorporate a square-edge optic feature to inhibit PCO.

The Effect of IOLs on Cell Growth and Modification of the Anterior and Posterior Capsule

The Hoya Vivinex IOL demonstrated a lag in cell growth across the posterior capsule following simulated cataract surgery relative to the Alcon Acrysof IOL. However, by day 28 (endpoint) the degree of coverage was similar, but the level of light scattering elements in the central visual axis was consistently greater with an Alcon Acrysof implanted than a Hoya Vivinex. Overall, it would appear that progression through different phases of PCO is retarded to some degree with the Hoya Vivinex IOL relative to Alcon Acrysof. These differences first seem apparent with progression of cells from the equatorial region onto the posterior capsule. This trend cannot be attributed to differences in the remaining anterior epithelium as remaining populations were comparable in both preparations from an individual donor. It is therefore likely that a design feature of the Hoya Vivinex IOL affords some biological benefit. The cells growing on the posterior capsule of Alcon Acrysof implanted bags typically encounter the IOL edge more rapidly than with Hoya Vivinex and movement beyond the square-edge is again more likely to occur with an Acrysof IOL implanted. The square-edge barrier afforded by the Hoya Vivinex does not appear to offer any more or less impedance to cell movement than the Alcon Acrysof counterpart. This suggests that square-edge barrier alone may not be sufficient to prevent PCO. With time a general pattern of cover on the central PC is established that is similar in both cases, but matrix contraction is significantly more advanced with Alcon

FIGURE 7. (**A**) Observation of cell outgrowth on the anterior IOL surface (central visual axis) in capsular bag preparations implanted with a Hoya Vivinex or Alcon Acrysof IOL. The data represent observations from 6 match-paired capsular bag experiments. At day 7, Hoya Vivinex samples do not show any cell growth on the anterior IOL surface (central visual axis). (**B**) Representative bright-field images showing cell coverage on Hoya Vivinex and Alcon Acrysof IOLs isolated from capsular bags at endpoint (28 days) and stained with Coomassie brilliant blue. (**C**) Pooled data showing coverage of the anterior IOL surface of Hoya Vivinex and Alcon Acrysof IOLs isolated from capsular bag preparations at endpoint (28 days). Data are presented as mean ± SD ($n = 6$). *Indicates a significant difference between groups (Student's *t*-test; $P \leq 0.05$).

Acrysof IOLs implanted. It would therefore seem that the Hoya Vivinex IOL tempers the first phase of cell growth immediately following surgery, which ultimately reflects a retardation of PCO changes over time relative to the Alcon Acrysof.

Cell Growth on the IOL

The ability of cells to grow on the anterior surface of both IOLs implanted was observed over the period of study. However, cells were more likely to migrate onto the Alcon Acrysof IOL than the Hoya Vivinex. To this effect, the first observations of cell growth on the Alcon Acrysof preceded those observed on the corresponding match-paired Vivinex IOL. Moreover, the extent of growth on the IOL was significantly more pronounced with an Alcon Acrysof IOL implanted than the Hoya Vivinex. This is an important feature as it has been previously reported that cells present on the IOL surface can cause light scatter[18] and thus contribute to visual deterioration following cataract surgery.

Previous reports have proposed that lens cells and capsule can establish an interaction with the IOL that influences its physical properties.[19,20] This has been implied for both the Alcon Acrysof and the Hoya Vivinex. In the latter case it has been suggested that UV/Ozone treatment of the posterior IOL surface increases interaction with cells using fibronectin as a biological glue.[21] The data obtained in our study suggests that interaction between lens cells and either of the IOLs tested is limited. When the cells have a choice of capsule or an IOL material they preferentially choose lens capsule as the native capsule provides complex biological signals that modulate cell behavior compared to a bioinert synthetic surface. Consequently, the major site of cell attachment on the IOLs studied was on the anterior surface of the optic within the central visual axis.

Future Developments

While a valuable tool for lens and IOL research, the newly described graded culture capsular bag model still possesses some limitations and has scope for further development. Elschnig's pearl and Soemmering's ring formation are not observed in the model.[8] Therefore, the results reported here present a fibrotic PCO model. Elschnig's pearls and Soemmering's ring typically develop at later stages in PCO development, and are generally believed to be associated with regenerative PCO. Future studies will aim to develop culture regimes to promote regenerative PCO within the capsular bag model. The model is also limited to some degree by the availability of tissue, but the opportunity to perform match-paired experiments greatly increases experimental power as two lenses from a single donor respond in a similar manner.[22] The graded culture regime utilized relatively high serum and TGFβ levels to initially drive the system followed by a gradual reduction and ultimately a return to serum free conditions. This allows key measures to be obtained in a limited experimental period, (i.e., 4 weeks), which permits more rapid feedback and will inform and facilitate IOL development more efficiently. The rate of change associated with the current graded culture regime is most likely to be greater in the model than in patients. As a result, the observed differences in the model could serve as a predictive tool of the relative rate and severity of PCO that may occur in a patient with different IOLs implanted during cataract surgery. The serum and TGFβ levels of the model could, be adapted further when clinical data of serum and TGFβ levels in patient are collected, and the culture period could be extended depending on the objective of the assay to measure a true time-progression or accelerated PCO model. Using this approach, a graded culture system to measure PCO formation will complement current research using clinical data and in vivo rabbit models.

Summary

The graded culture human capsular bag model reflects the pattern of postsurgical changes in the ocular environment and gives rise to clinical features of PCO. The truncated timeframe of 1 month (28 days cell culture) enables IOLs to be assessed for PCO formation tendency, as opposed to implanting the IOLs in a rabbit model in which PCO is aggressive or to implanting the IOLs in human patients and waiting for postmortem assessment of PCO performance. It therefore serves as an excellent system to evaluate and develop intraocular lenses. Using this system, it was found that the Hoya Vivinex IOL showed an overall better level of performance than the Alcon Acrysof IOL, such that progression of PCO appears to be slower. In addition, cells are less likely to populate the IOL surface of the Hoya Vivinex IOL than the Alcon Acrysof IOL, which will further reduce light scatter following surgery.

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