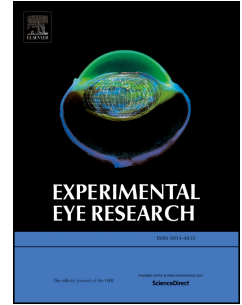


Journal Pre-proof

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PII: S0014-4835(21)00052-X

DOI: <https://doi.org/10.1016/j.exer.2021.108487>

Reference: YEXER 108487

To appear in: *Experimental Eye Research*

Received Date: 11 December 2020

Revised Date: 22 January 2021

Accepted Date: 1 February 2021

Please cite this article as: Wormstone, I.M., Damm, N.B., Kelp, M., Eldred, J.A., Assessment of intraocular lens/capsular bag biomechanical interactions following cataract surgery in a human in vitro graded culture capsular bag model, *Experimental Eye Research* (2021), doi: <https://doi.org/10.1016/j.exer.2021.108487>.

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**Assessment of intraocular lens/capsular bag biomechanical interactions
following cataract surgery in a human in vitro graded culture capsular bag
model**

I. Michael Wormstone^{1*}, Niklas B. Damm², Martin Kelp², Julie A Eldred¹

¹School of Biological Sciences, University of East Anglia, Norwich, NR4 7TJ; ²ZEISS
Ophthalmic Devices, Carl Zeiss Meditec AG, 10589 Berlin, Germany

Corresponding Author*

Michael Wormstone PhD FARVO
Professor of Ophthalmology
School of Biological Sciences
University of East Anglia,
Norwich Research Park,
Norwich, NR4 7TJ

Tel: +44 (0)1603 592781

Email: i.m.wormstone@uea.ac.uk

Highlights

- The human graded culture capsular bag model can be studied for three-months
- The model presents features of both fibrotic and regenerative PCO
- Biomechanical interaction can be measured and compared over time using the model
- The CT LUCIA 611PY IOL is stable within the bag and has a strong optic barrier effect
- Optic barrier results from anterior/posterior capsule fusion in the peripheral bag

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Abstract

Intraocular lenses (IOLs) are implanted during cataract surgery. For optimum results, stable positioning of the IOL in the capsular bag is important. Wound-healing events following cataract surgery lead to modification of the capsular bag and secondary visual loss due to posterior capsule opacification. At present, it is unclear how these biological events can affect stability of the IOL within the capsular bag. In the present study, a human in vitro graded culture capsular bag model was the experimental system. Capsulorhexis and lens extraction performed on human donor eyes generated suspended capsular bags (5 match-paired experiments). Preparations were secured by pinning the ciliary body to a silicone ring and maintained in 6 mL of medium for 84 days using a graded culture system: days 1–3, 5% human serum and 10 ng/mL transforming growth factor β (TGF β 2); days 4–7, 2% human serum and 1 ng/mL TGF β 2; days 8–14, 1% human serum and 0.1 ng/mL TGF β 2; days 15–84, serum-free Eagle's minimum essential medium (EMEM). A CT LUCIA 611PY IOL was implanted in all preparations. Quantitative measures were determined from whole bag images captured weekly. Images were registered using FIJI and analysed in ImageJ to determine capsular bag area; distortion; angle of contact; haptic stability; capsulorhexis area; and a fusion footprint associated with connection between the anterior and posterior capsules. Cell coverage and light scatter were quantified at end-point. The transdifferentiation marker, α -SMA was assessed by immunocytochemistry. Immediately following surgery, distortion of the capsular bag was evident, such that a long axis is generated between haptics relative to the non-haptic regions (short axis). The angle of contact between the haptics and the peripheral bag appeared inversely correlated to capsular bag area. Growth on the peripheral posterior capsule was observed 1 week after surgery and beneath the IOL within 1 month. As coverage of the posterior capsule progressed this was associated with matrix contraction/wrinkles of both the central posterior capsule and peripheral capsular bag. Cells on the central posterior capsule expressed α SMA. Fusion footprints formed in non-haptic regions of the peripheral bag and progressively increased over the culture period. Within and at the edge of the fusion footprint, refractive structures resembling lens fibre cells and Elschnig's pearls were observed. Cell attachment to the IOL was limited. An impression in the posterior capsule associated with the CT LUCIA 611PY optic edge was evident; cell density was much greater peripheral to this indent. Wound-healing events following surgery reduced capsular bag area. This was associated with the long/short axis ratio and angle of contact increasing with time. In summary, we have developed a human capsular bag model that exhibits features of fibrotic and regenerative PCO. The model permits biomechanical information to be obtained that enables better understanding of IOL characteristics in a clinically relevant biological system. Throughout culture the CT LUCIA 611PY appeared stable in its position and capsular bag modifications did not change this. We propose that the CT LUCIA 611PY optic edge shows an enhanced barrier function, which is likely to provide better PCO management in patients.

Keywords: cataract surgery; intraocular lens; posterior capsule opacification; human; lens; in vitro; model; capsular bag.

1.0. Introduction

Intraocular lenses (IOLs) are routinely implanted during cataract surgery to restore optical power and improve post-operative visual outcomes (Perez-Vives, 2018; Wormstone et al., 2009; Wormstone et al., 2020). To achieve optimum results, positioning of the IOL is important. Optical performance can be affected by a number of factors, such as tilt, decentration and IOL rotation; the latter is particularly important in relation to toric IOLs (Kimura et al., 2017). At the time of surgery, the IOL is introduced into the capsular bag and at this point, its position is largely governed by physical interactions between the IOL and the capsular bag. Effort is made to position the IOL, but inevitably, perfect alignment with minimal tilt is not always achievable. However, visual acuity tests suggest initial outcomes are still excellent (Toyama et al., 2018) .

In order to meet international standards for IOL manufacture a number of tests are performed to predict IOL behaviour when implanted. These ISO guided tests take place in a non-biological setting and are limited to assessing purely physical characteristics. This information is certainly valuable, but no in vitro tests are routinely performed that consider the biological influence on IOL stability and performance.

Following surgery, residual lens epithelial cells that have survived the rigours of the operation embark upon a wound healing response, such that cells can grow on capsular surfaces and in some cases the IOL (Wormstone et al., 2009). This growth is often associated with fibrotic events such that a number of cells can transdifferentiate into myofibroblasts, increase matrix production and cause contraction of the matrix (Eldred et al., 2011; Wormstone et al., 2009; Wormstone et al., 2020). In addition, attempts to regenerate a new lens through differentiation are observed in the form of Soemmerring's ring and Elschnig's pearls (Wormstone et al., 2009). Together these events contribute to light scatter and significant visual deterioration. This condition is known as posterior capsule opacification or after-cataract, which requires Nd:YAG laser capsulotomy to correct (Wormstone et al., 2009; Wormstone et al., 2020).

The cellular modification of the capsular bag is important and can potentially increase tilt, IOL decentration and rotation. Cell induced interactions with the capsule and IOL have also been cited as a beneficial mechanism to prevent PCO formation (Linnola, 1997; Linnola et al., 2000a, b). Some years ago, Linnola proposed the sandwich theory to explain how square-edge IOLs can provide better PCO management than round-edge IOLs (Linnola, 1997). This concept argues that the IOL material can be bioactive, allowing the cells to adhere to the IOL and posterior capsule, resulting in a sandwich of cells between these surfaces. It is proposed that acrylic IOLs form a stronger seal with the capsule through cell

interactions and fibronectin deposits (Linnola et al., 2000a, b). This theory proposes that a tight seal is formed between the optic edge and the capsule, which provides a barrier to impede further cell movement onto the central posterior capsule and thus lower PCO incidence with square-edge IOLs.

Understanding events that take place following cataract surgery is important in terms of IOL stability and PCO management. The current study aimed to determine whether or not extending the experimental time period of the graded culture human capsular bag model would reveal previously unreported features of regenerative PCO. Additionally, we aimed to use this platform to establish quantitative analysis of IOL stability within a biological and clinically relevant system, thus providing knowledge and approaches that have previously been unavailable in IOL development. The method described in the current study provides valuable insight regarding IOL interaction with the capsular bag that is impossible to predict in the absence of a biological system. Moreover, the work sheds new light on the sequence of events following surgery, which suggests that a modification of the sandwich theory for PCO prevention is required.

2.0. Methods

2.1. The Capsular bag model

The model previously described by Liu et al (Liu et al., 1996), developed by Cleary et al (Cleary et al., 2010) and further refined by Eldred et al (Eldred et al., 2014; Eldred et al., 2019) was employed (Wormstone, 2020). 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. A step-vaulted hydrophobic acrylic 24.0 dioptre ZEISS CT LUCIA 611PY IOL (optic diameter 6.0mm; total diameter Ø13.0mm) was implanted in every capsular bag. The capsular bag (with 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. Preparations were maintained in defined culture conditions over 84 days. To better reflect the clinical changes to the lens environment post-surgery (Eldred et al., 2011; Pande et al., 1996; Wormstone et al., 2020), a previously reported graded culture system was employed (Eldred et al., 2019). The culture regime was as follows: Days 1-3, 5% Human serum and 10ng/ml TGFβ₂; Days 4-7, 2% Human serum and 1ng/ml TGFβ₂; Days 8-14, 1% Human serum and 0.1ng/ml TGFβ₂; Days 15-84, Serum-free EMEM. Low-power modified darkfield microscopy captured whole bag images weekly along with higher power phase-contrast microscopy over a 12-week period.

2.2. Quantitative measures from time-lapse whole bag images

Quantitative measures of the capsular bags over time were determined from whole bag images capture weekly from Day 0 to day 84. To achieve this, images were uploaded in FIJI (Fiji/ImageJ) and registered using the TrakEM2 feature. The images were stacked sequentially from Day 0 to 84 and registered manually against the base section of one haptic. The stacked and registered images were analysed in ImageJ 1.48v (<http://imagej.nih.gov/ij/> provided in the public domain by the National Institutes of Health, Bethesda, MD, USA), to determine: capsular bag area; distortion through measurements of a long and short axis of the capsular bag; angle of contact between the haptics and equator of the capsular bag; haptic stability through changes in tip-to-tip and notch-to-notch length; capsulorhexis area; a fusion footprint associated with connection between the anterior and posterior capsules (See Figure 1 for illustrative examples).

2.3. End-point analysis

2.3.1. Quantification of cell cover

The level of cell coverage on the posterior capsule (PC), within the visual axis, was quantified using FIJI analysis software (Fiji/ImageJ). To achieve this, low-power dark-field images of capsular bags with IOLs removed captured at end-point were analysed, such that the central visual axis (defined 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.

2.3.2. Quantification of light scatter

To establish a measure of light scatter in the central PC, ImageJ 1.48v (<http://imagej.nih.gov/ij/> provided in the public domain by the National Institutes of Health, Bethesda, MD, USA) was employed. Images were converted to 8-bit greyscale images and subjected to the find edges function, which identifies light scattering regions. These regions appear bright, and thus have a high intensity per pixel relative to non-scattering regions. The images were then thresholded to provide a binary image, such that light scattering regions appear as black pixels. The rhexis area is defined and the black pixel number relative to the rhexis area quantified.

2.3.3. Immunocytochemistry

Transdifferentiation of epithelial cells to myofibroblasts is a clinical feature of PCO. We, therefore, employed immunocytochemistry to visualise and quantify expression of the myofibroblast marker, α -SMA. All reagents were from Sigma (Poole, Dorset, UK) unless otherwise stated. Washes were for 3 x 15 min in phosphate buffered saline (PBS). IOLs were removed from capsular bag preparations then fixed for 30 min in 4% formaldehyde in PBS and permeabilised in PBS containing 0.5% Triton-X100, also for 30 min. Non-specific sites were blocked with normal goat serum (1:50 in 1% bovine serum albumin/PBS). Anti-alpha smooth muscle actin and anti-fibronectin was diluted 1:100 in 1% bovine serum albumin/PBS and applied for 60 min at 35 °C, followed by washing. Alpha smooth muscle actin and fibronectin presence was visualized with ALEXA 488-conjugated secondary antibodies (Molecular Probes, Leiden, Netherlands). Samples were then counterstained with 4',6-diamidino-2-phenylindole (DAPI) and Texas red-x-phalloidin (Invitrogen). The stained

preparations were again washed extensively, floated onto microscope slides and mounted in Hydromount mounting medium (National Diagnostics, Hull, UK). Images were viewed with a Zeiss epifluorescence microscope and Zeiss Axiovision software.

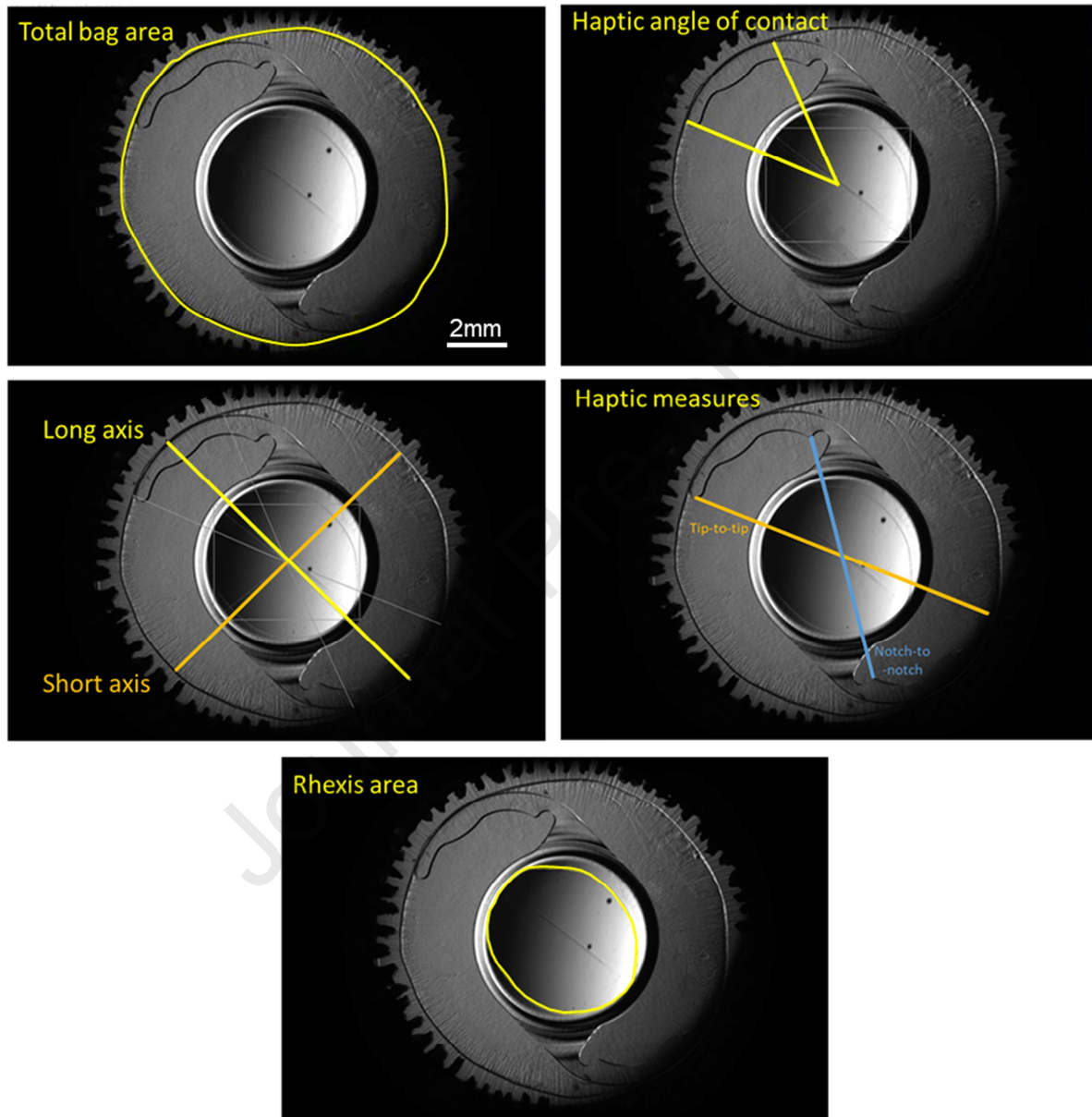


Figure 1. Illustrative examples highlighting quantitative measures derived from low-power images of the entire capsular bag.

3.0. Results

3.1 Comments on surgeries

Ten eyes from five donors (Range 50-82 years; Mean Age 69.4 ± 13.2 years; 3 male and 2 Female) were used in this study (Table 1). A capsulorhexis ~ 5 mm in diameter was performed on the first 2 donors. Due to the shape of the ZEISS CT LUCIA 611PY IOL, at the peripheral part of the optic, this sometimes made it difficult to see the edge of the rhexis down the microscope. In response to this, a smaller capsulorhexis (~ 4.5 mm diameter) was performed on donors 3-5 to enable better assessment of the rhexis margin (Figure 2). In all cases the capsulorhexis was completely mounted on the anterior IOL optic face (Figure 3). Cell viability of the anterior lens epithelium following simulated cataract surgery was comparable between each capsular bag preparation from a single donor. While differences in retention of the anterior epithelium did vary from donor to donor, in all cases viable cells remained.

	Age (years)	Gender
Donor 1	72	Male
Donor 2	82	Female
Donor 3	63	Female
Donor 4	50	Male
Donor 5	80	Male

Table 1. Donor details

3.2. Assessment of physical measures immediately following surgery

Whole bag images captured immediately following surgery (Figure 3) were analysed to provide reference values for quantitative measures and allow intra- and inter-donor comparison. It is notable that the capsular bag size differs from donor to donor, but is similar within a donor pair (Figure 4). It is clear that introduction of the CT LUCIA 611PY IOL imposes a loss of circularity of the capsular bag, such that a long axis is generated between haptics relative to the non-haptic regions, which present a short axis (Figure 4). This pattern can be expressed as a long/short axis ratio and serves as useful measure of circularity/distortion (Figure 4). The average ratio for the five donors was 1.062 ± 0.0029 . This value was relatively uniform across donors and did not correlate with the size of the capsular bag (Figure 5). Interestingly, the angle of contact between the haptics and the peripheral bag did again differ between donors, but remained similar within a donor pair. This angle of contact appeared inversely correlated to capsular bag area. Notch-to-notch

measurements between haptics were relatively constant between donors, as predicted, but tip-to-tip length varied between donors and correlated with capsular bag area (Figure 5).

3.3. Biological observations over time

In the early stages of culture, growth on the capsular bag followed a similar pattern to previous studies (Eldred et al., 2019). Following a 1 week culture period, cells had recolonised denuded regions of the anterior capsule and showed signs of growth on the peripheral posterior capsule (Figure 6A). By day 14, 2 out of 5 donors presented cells growing on the posterior capsule within the central visual axis (within the rhexis margin; Figures 6A & B). A week later (Day 21), cells were observed on the central posterior capsule of all preparations (Figures 6A & B). Viable cells appeared to be present on all preparations at end-point (Day 84) and mean coverage of the central posterior capsule was $66.3 \pm 8.7\%$ (Range: 37.4 – 85.8%) for the 5 donors. As coverage of the posterior capsule progressed this was associated with matrix contraction/wrinkles of both the central posterior capsule and peripheral capsular bag (Figures 6A & C). Mean light scatter score was 0.042 ± 0.014 (Range: 0.020 – 0.058). End-point analysis using fluorescence microscopy demonstrated that cells expressed α SMA (a marker of myofibroblasts), fibronectin (a fibrotic matrix marker), had a dynamic F-actin distribution and presented multi-layered nuclei (Figure 6D). Growth was also observed on the IOL of some of the preparations. Four donors presented some cell growth on the anterior IOL surface within the first month of culture, but this outgrowth regressed over time (Figure 7A). Cells were also observed growing on the posterior IOL surface of three donors (Figure 7B).

Another interesting feature observed and quantified was the process of cell-mediated fusion of the anterior and posterior capsules. This process occurred in all preparations. An example of this phenomenon can be seen in Figure 8. We term the degree of fusion at any point in time a fusion footprint. In the early stages of formation, definition can be difficult to establish, but becomes clearer and easier as it progresses. When considering all donors, a significant difference from control was observed at day 49. The extent of the fusion footprint continued to increase beyond this point. It is notable that a fusion footprint is established in the non-haptic regions of the bag. It would appear that the haptic and optic maintain separation of the anterior and posterior capsules, thus preventing fusion at these sites.

Extending the culture period beyond 28 days to 84 days was revealing. In addition to connecting the anterior and posterior capsules, the fusion zone is a site of dynamic change. At the edge of the fusion footprint, formation of refractive structures was observed (Figures 9A, B and C). End-point investigations at the edge of the fusion footprint in one donor

revealed an F-actin organisation that resembled lens fibre cells indicating de novo differentiation is taking place in these capsular bag cultures (Figure 9D & E). Unusual cells were also seen in the haptic region i.e. where fusion had not taken place. Here “balloon” cells could be observed (Figure 9F-J). These cells seemed to be tethered to the capsule at a specific point, while the bulk of the cell floated in the medium between the capsules. These cells appeared to contain a nucleus.

Following removal of the IOL at end-point, it was apparent that the IOL had interacted with the capsule to establish a barrier in association with the optic edge. This did not prevent cell growth on the posterior capsule, but it was apparent that cells beneath the optic were larger and showed a reduced density relative to the tight compact cells peripheral to the optic (Figure 10).

3.4. Impact of biological events on quantitative measures

Analysis of day 0 capsular bags demonstrated physical differences between donors. In order to overcome these differences and assess relative change over time, data were normalised and expressed as % T = 0. Culture of capsular bags led to a reduction in area within the first week of culture. This reduction continued slowly, but progressively over time (Figure 11). The mean reduction in area at end point was $4.26 \pm 0.45\%$ of control. This was associated with a reduction in the short axis i.e. across the non-haptic regions, which decreases by $3.92 \pm 0.50\%$, while the long axis remained stable throughout (Figure 11). Consequently, the long/short axis ratio increased with time by $4.17 \pm 0.45\%$ and was fundamentally determined by changes in the short axis. The angle of contact also increased within a week to $108.46 \pm 1.31\%$ of values measured post-surgery (T=0) and continued to increase over time as further contraction of the bag took place (Figure 12); at day 84, the angle of contact was $115.67 \pm 2.26\%$ of T=0 values. The tip-to-tip and notch-to-notch lengths did not significantly change over time (Figure 12). The capsulorhexis significantly reduced in size by $2.49 \pm 0.50\%$ within the first week of culture before returning to its original size by week 3 followed by a slow and moderate reduction over time (Figure 13).

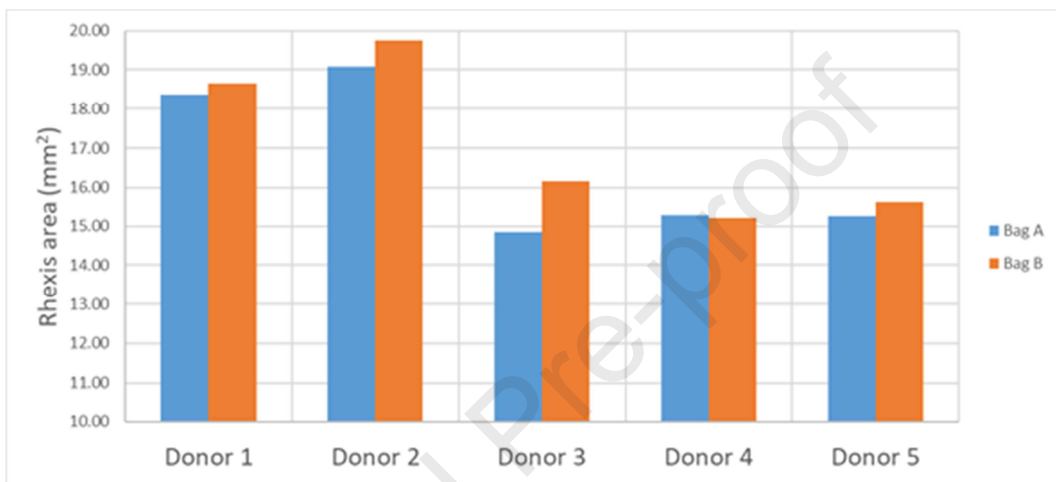


Figure 2. Quantitative assessment of the central opening, defined by the capsulorhexis, in all preparations shortly after surgery.

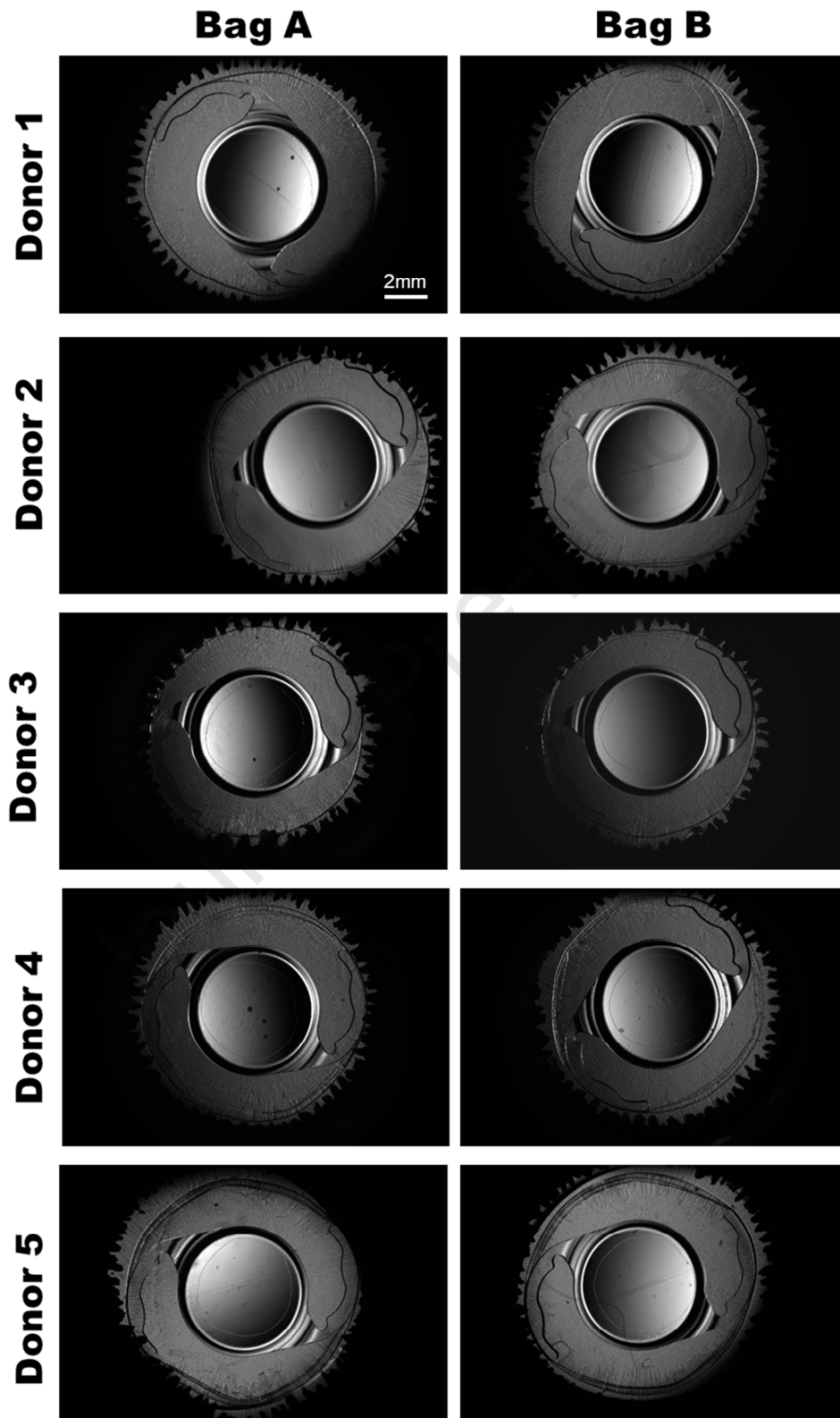


Figure 3. Modified dark-field images of capsular bags implanted with a ZEISS CT LUCIA 611PY IOL captured on the day of simulated surgery. In all cases, the capsulorhexis is fully seated on the IOL optic.

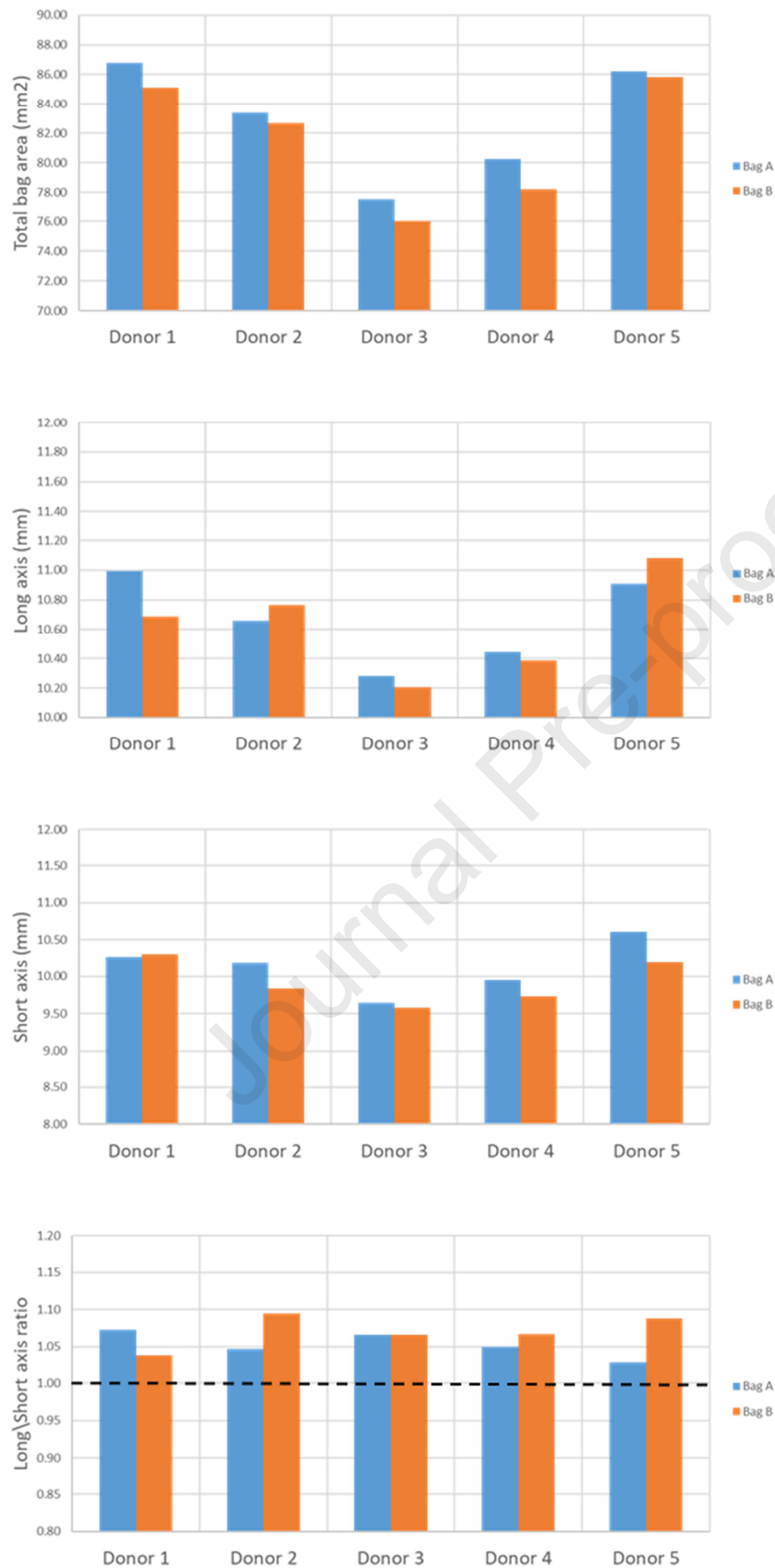


Figure 4. Quantitative assessment of the total capsular bag area and distortion following implantation of a ZEISS CT LUCIA 611PY IOL, in all preparations shortly after surgery.

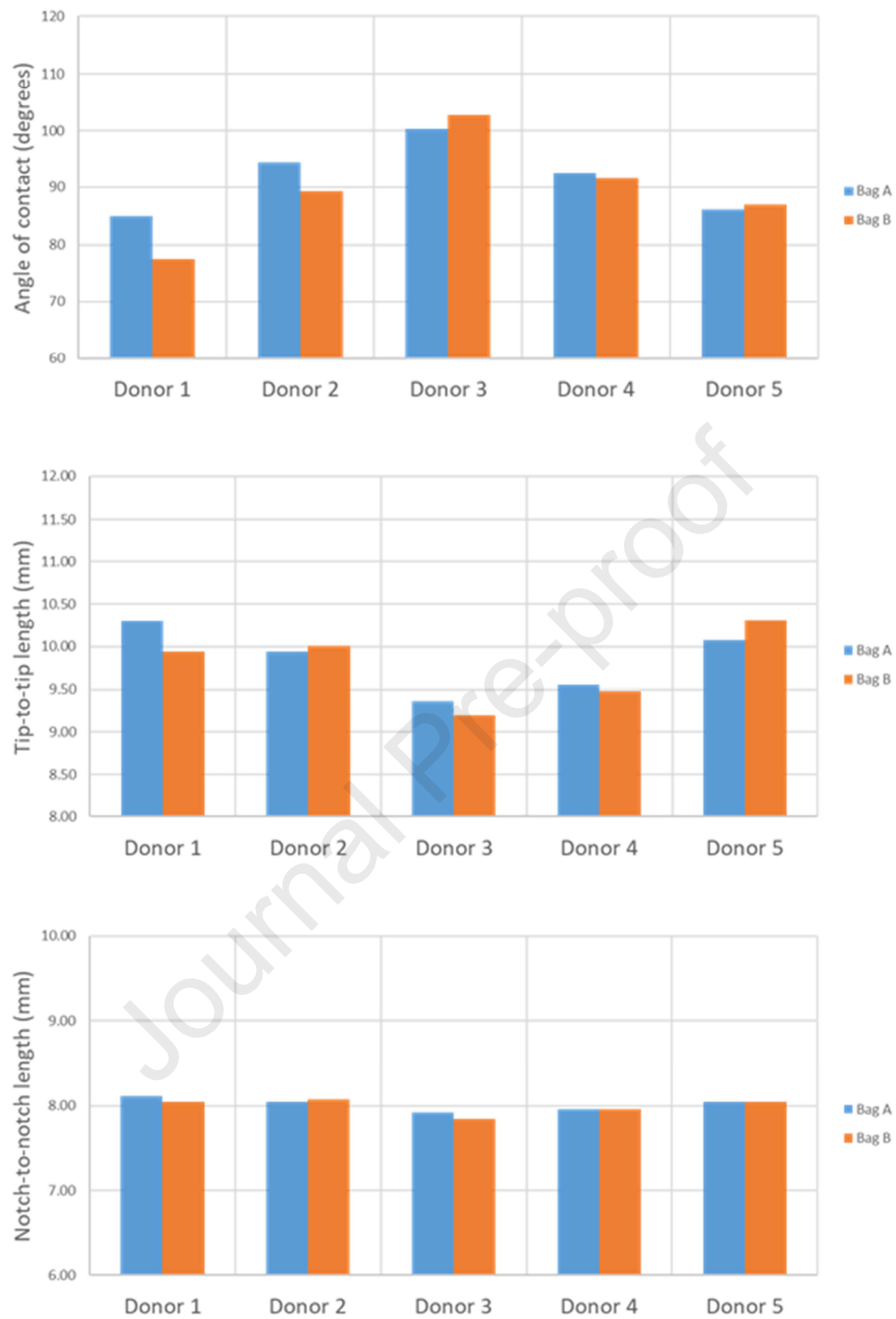
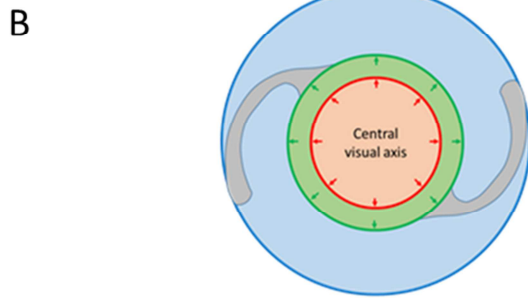
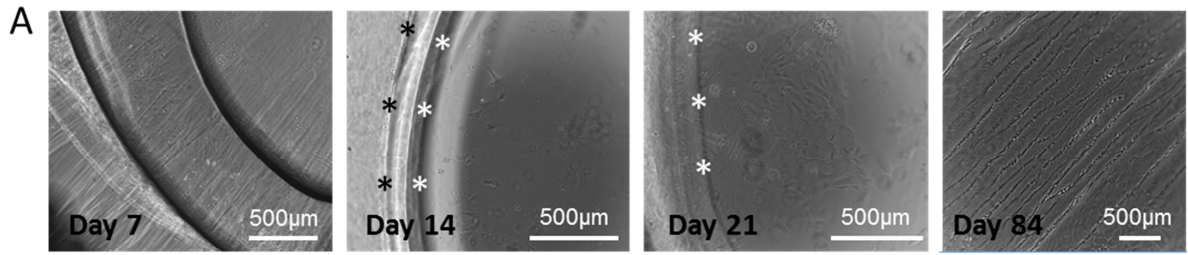
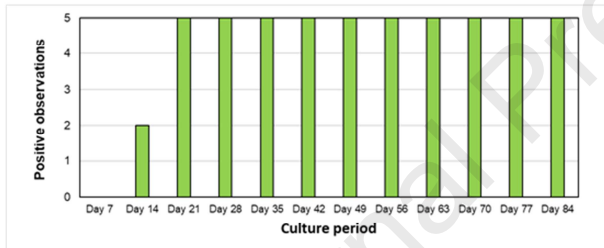


Figure 5. Quantitative assessment of the angle of contact established between the haptic and capsular bag equator and measures of haptic positioning (tip-to-tip length and notch-to-notch length) following implantation of a ZEISS CT LUCIA 611PY IOL, in all preparations shortly after surgery.



Optic-edge breached



Cells within central visual axis

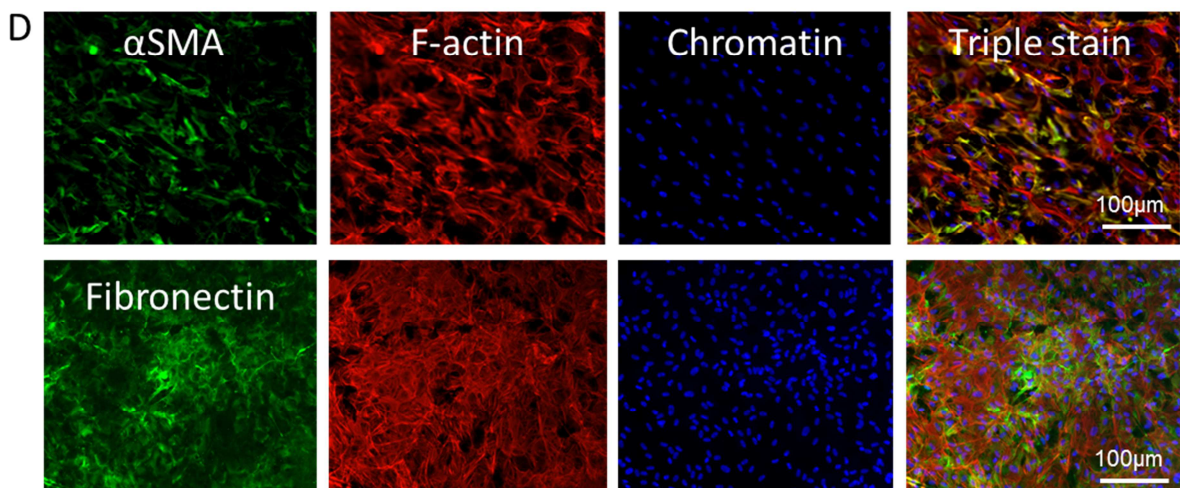
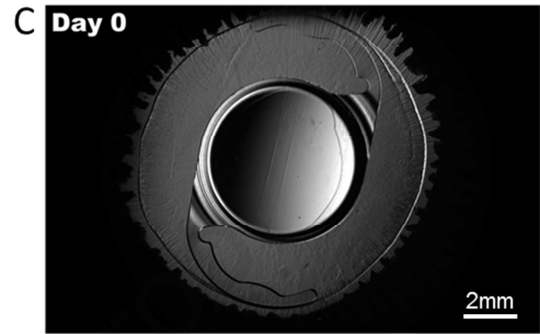
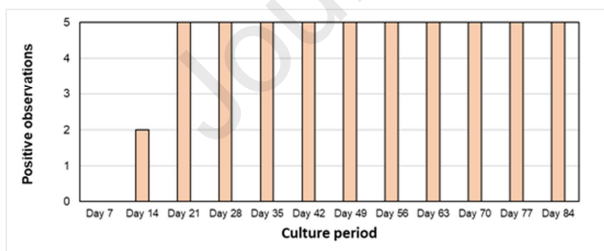


Figure 6. Assessment of growth characteristics and matrix modification in capsular bags. (A) Representative phase-contrast micrographs showing progressive cell growth on the posterior capsule of capsular bags implanted with a ZEISS CT LUCIA 611PY IOL. The capsulorhexis edge is highlighted by white asterisks and the IOL optic perimeter is highlighted by black asterisks. (B) Observation of cell growth progression on the posterior capsule in capsular bag preparations implanted with a ZEISS CT LUCIA 611PY IOL. The data represent observations from five donors. (C) Representative modified dark-field and phase-contrast images of capsular bags, captured shortly following surgery (Day 0), the 28-daytime point and endpoint (Day 84). (D) Representative epifluorescence micrographs showing the distribution of the myofibroblast marker, α -smooth muscle actin, the fibrotic matrix marker, fibronectin, F-actin and chromatin associated with cells growing on the central posterior capsule of human lens capsular bag preparations implanted with a ZEISS CT LUCIA 611PY IOL . Images were captured at endpoint (day 84).

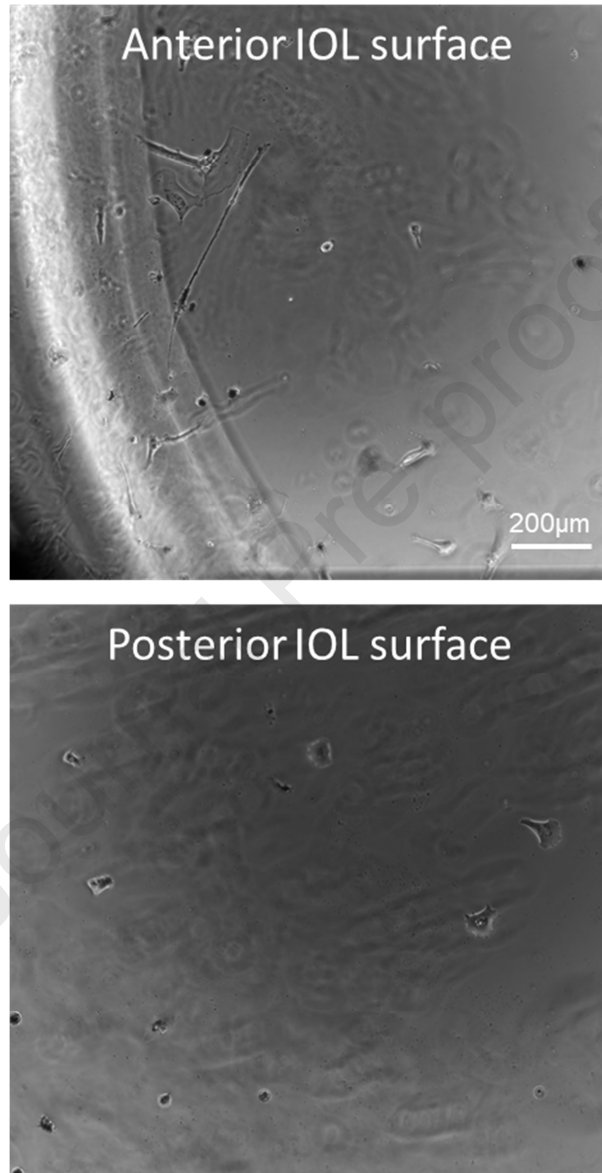


Figure 7. Illustrative examples of phase-contrast micrographs show movement/attachment of cells on to the anterior and posterior surface of the IOL.

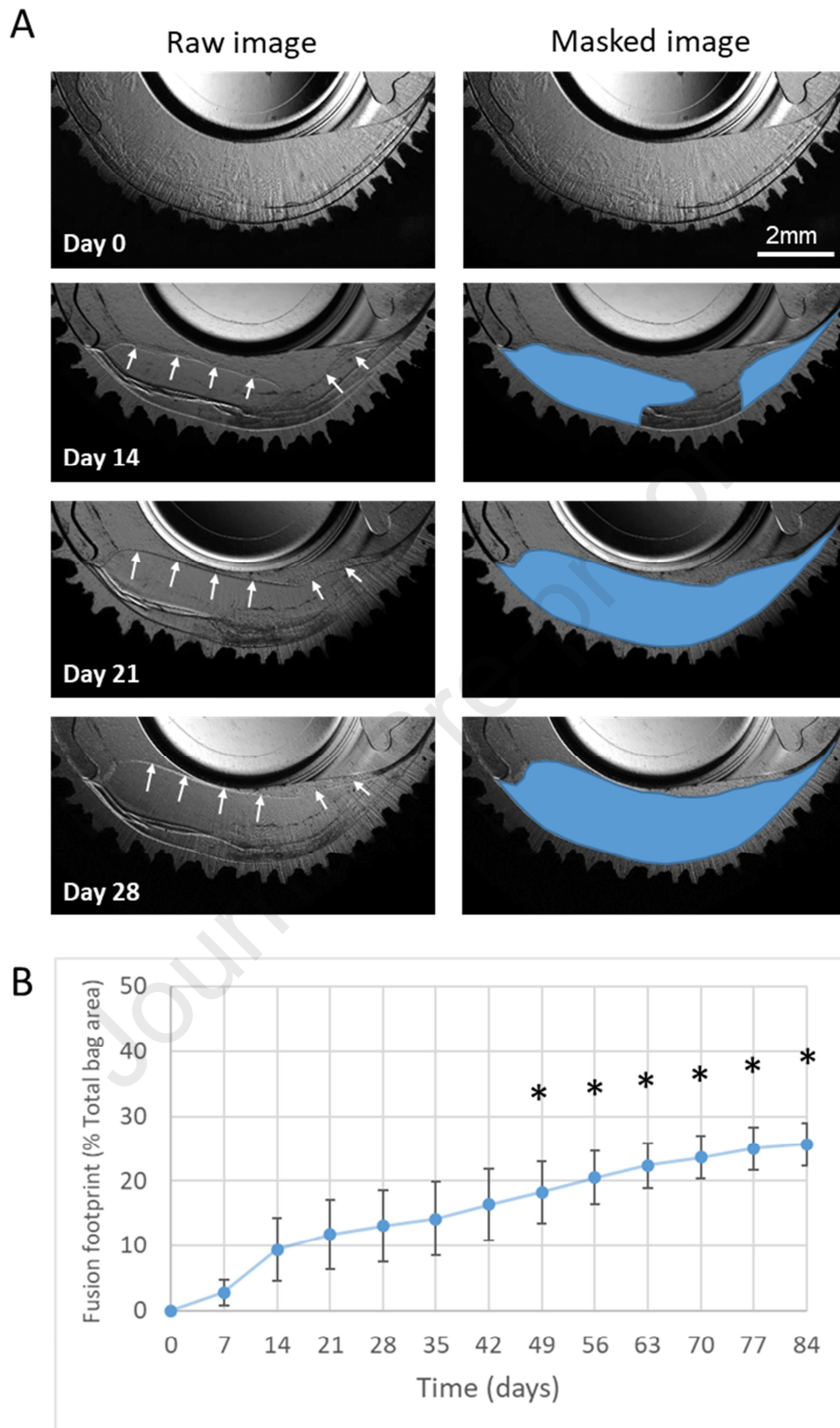


Figure 8. Anterior/posterior capsule fusion footprint within capsular bags implanted with a ZEISS CT LUCIA 611PY IOL. (A) Representative images with and without a mask highlighting fused regions, which show the progression of the fusion footprint over time. (B) Quantitative assessment of the fusion footprint over an 84-day culture period. The data are expressed as Mean \pm SEM (n=5). * indicates a significant difference from day 0 ($p \leq 0.05$; ANOVA with Dunnett's post hoc test).

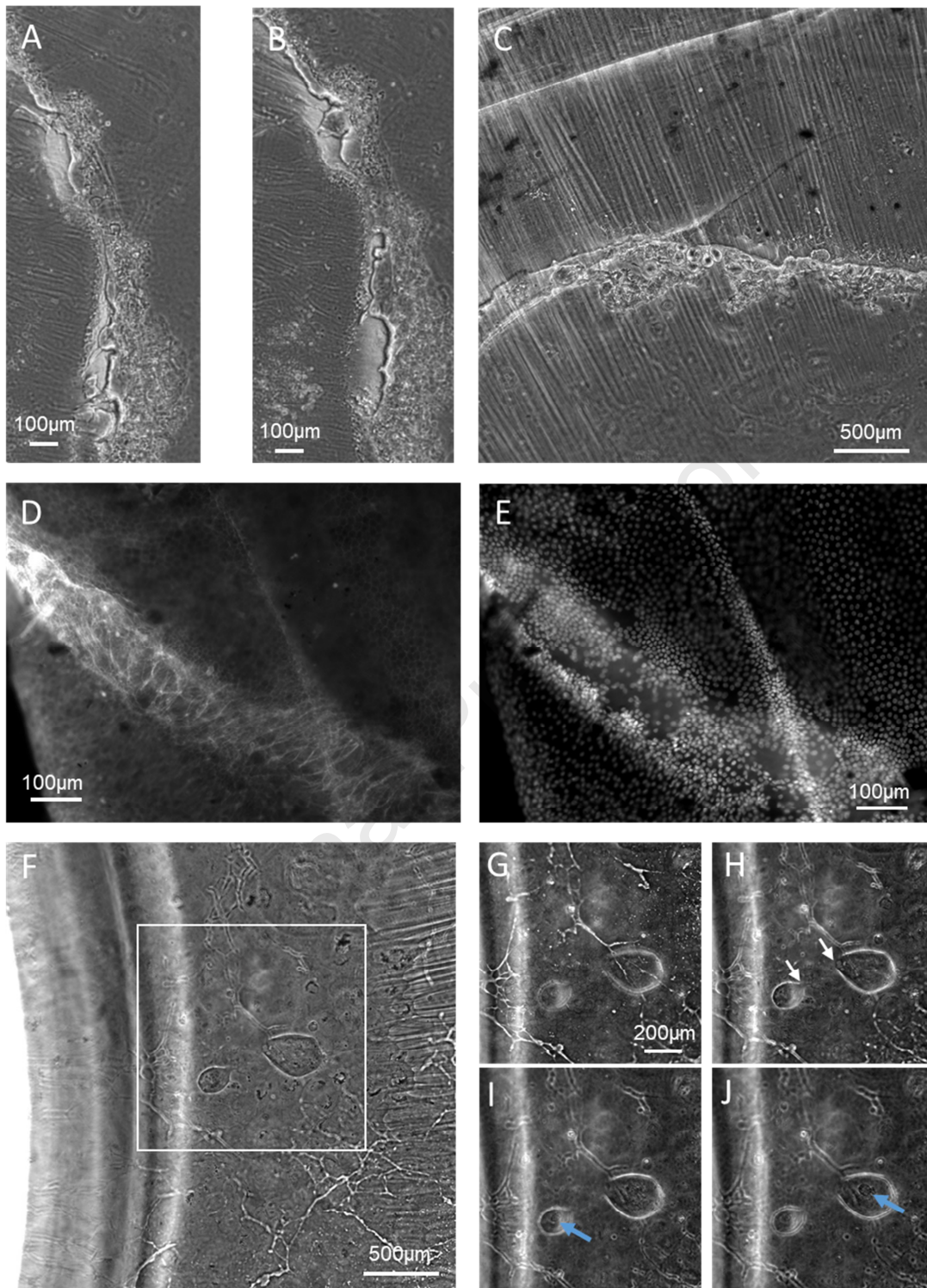


Figure 9. Formation of refractive structures and atypical cells in cultured capsular bags implanted with a ZEISS CT LUCIA 611PY IOL. (A & B) show the formation of refractive structures along the edge of the fusion footprint. Image A captured at Day 42 and B a week later. (C) Appearance of pearl-like structures at the edge of the fusion footprint. (D & E) Fluorescent micrographs captured at end-point (Day 84) showing F-actin organisation and corresponding chromatin/nuclei respectively. F-Actin appearance in the refractive areas is elongated and defined at the cell membrane, which resembles that associated with lens fibre cells. (F-J) “Balloon” like cells observed close to the IOL optic within the bay of the haptic i.e. non-fused region. G-J show different planes, which highlight a point at which these cells tether to the capsule (white arrows) and that they retain a nucleus (blue arrows).

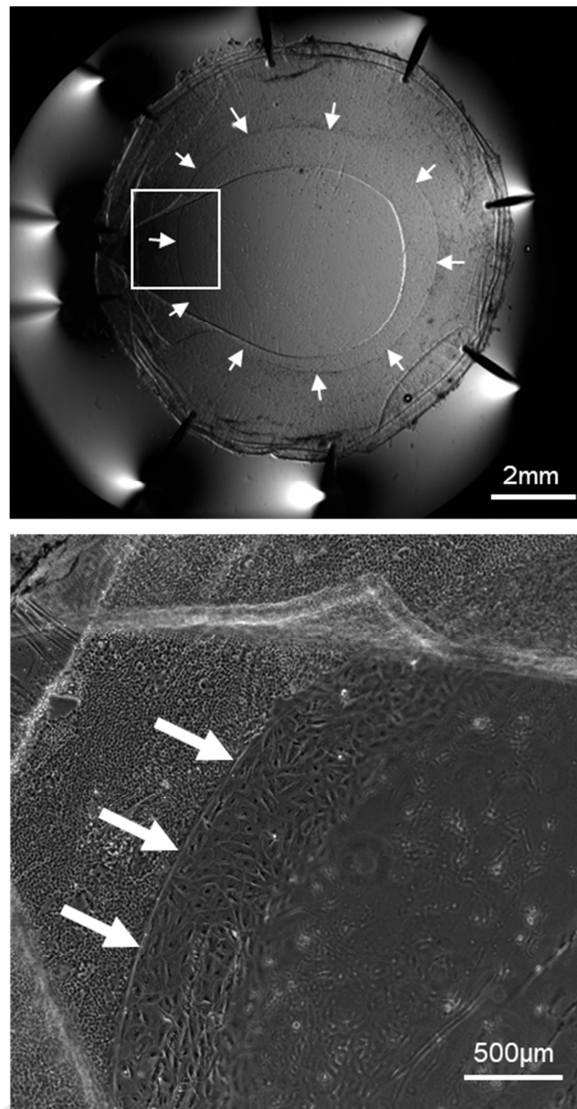


Figure 10. Evidence of interaction between the IOL edge and the posterior capsule. (A) A representative modified darkfield micrograph showing the entire capsular bag following IOL removal and fixation. The impression created in the capsule is clear to see (defined by white arrows) and appears to be 360°. This pattern was observed in all preparations. (B) Higher power phase-contrast micrograph illustrating the distinct impression created by the IOL and distinct differences in size and organisation either side of this divide.

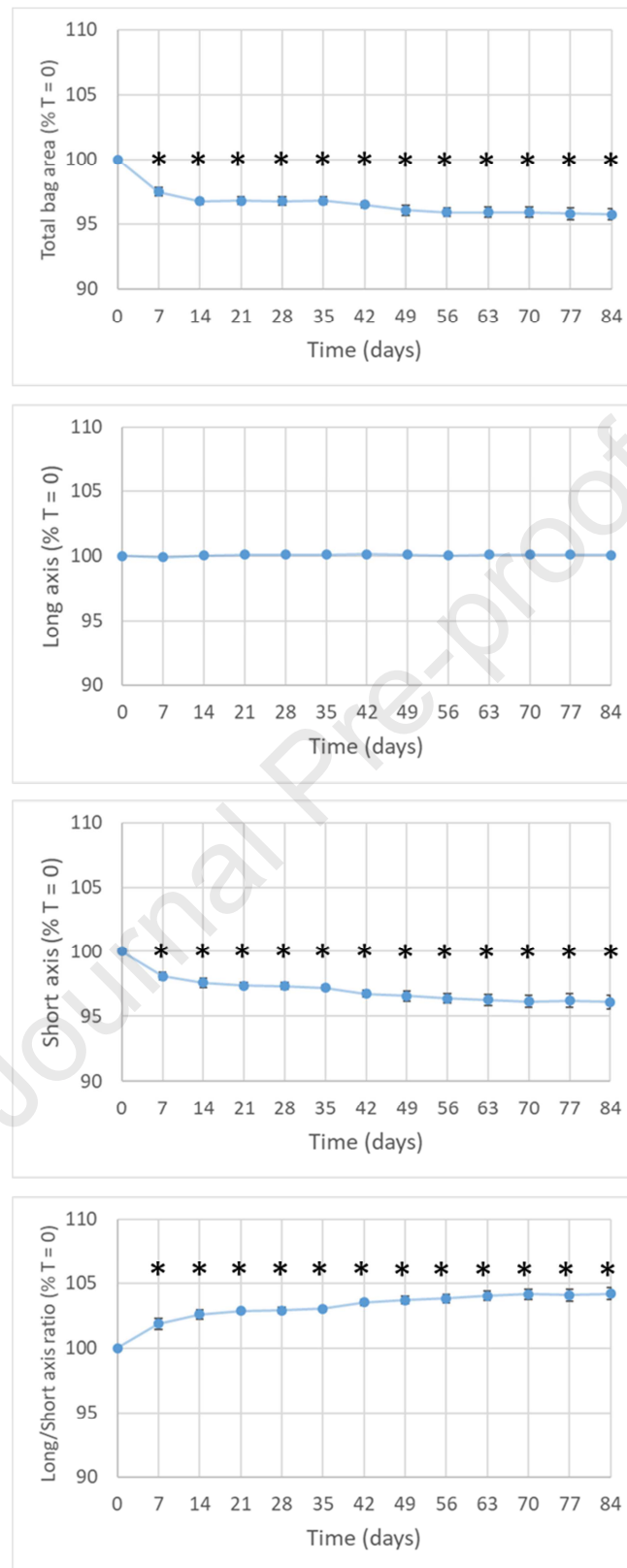


Figure 11. Quantitative assessment of the total capsular bag area and distortion following implantation of a ZEISS CT LUCIA 611PY IOL over an 84-day culture period. The data are expressed as Mean \pm SEM (n=5). * indicates a significant difference from day 0 ($p \leq 0.05$; ANOVA with Dunnett's post hoc test).

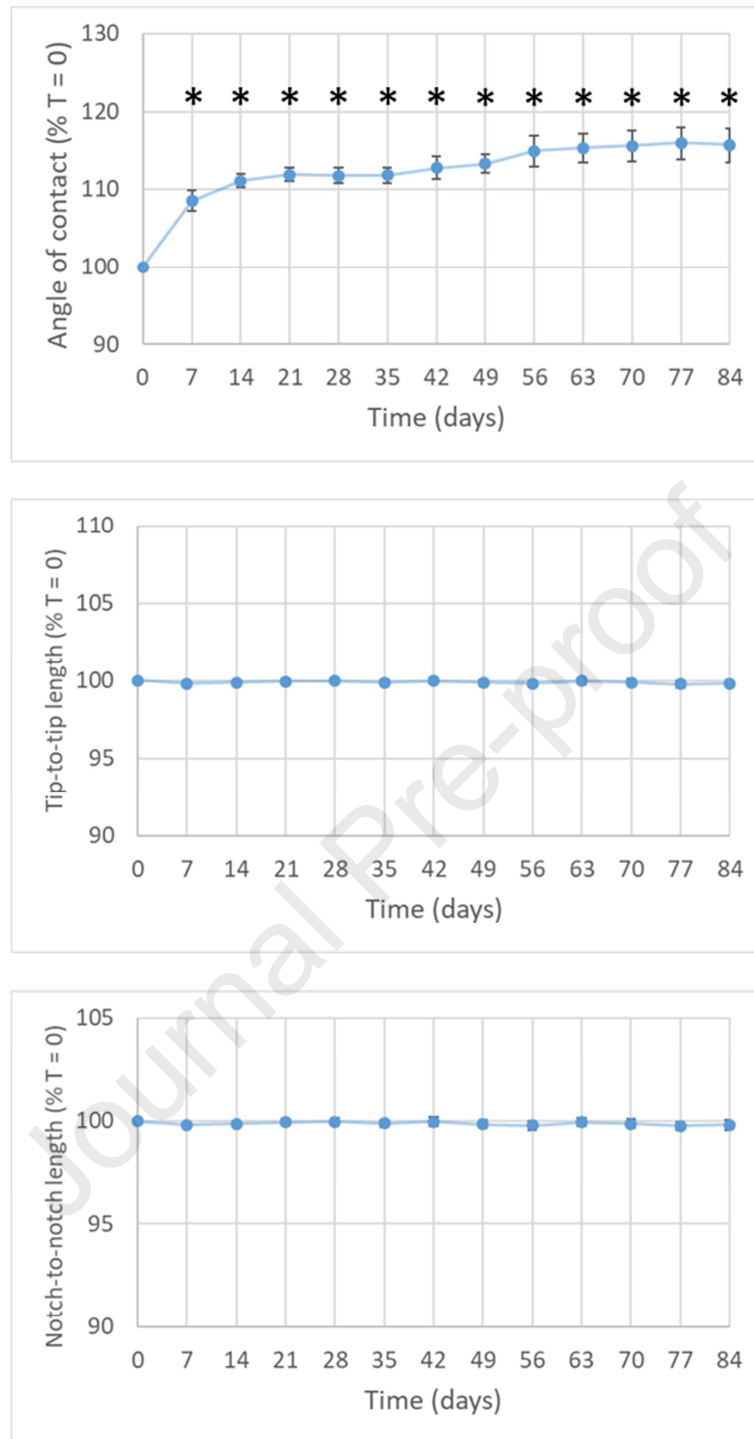


Figure 12. Quantitative assessment of the angle of contact established between the haptic and capsular bag equator and measures of haptic positioning (tip-to-tip length and notch-to-notch length) following implantation of a ZEISS CT LUCIA 611PY IOL over an 84-day culture period. The data are expressed as Mean \pm SEM (n=5). * indicates a significant difference from day 0 ($p \leq 0.05$; ANOVA with Dunnett's post hoc test).

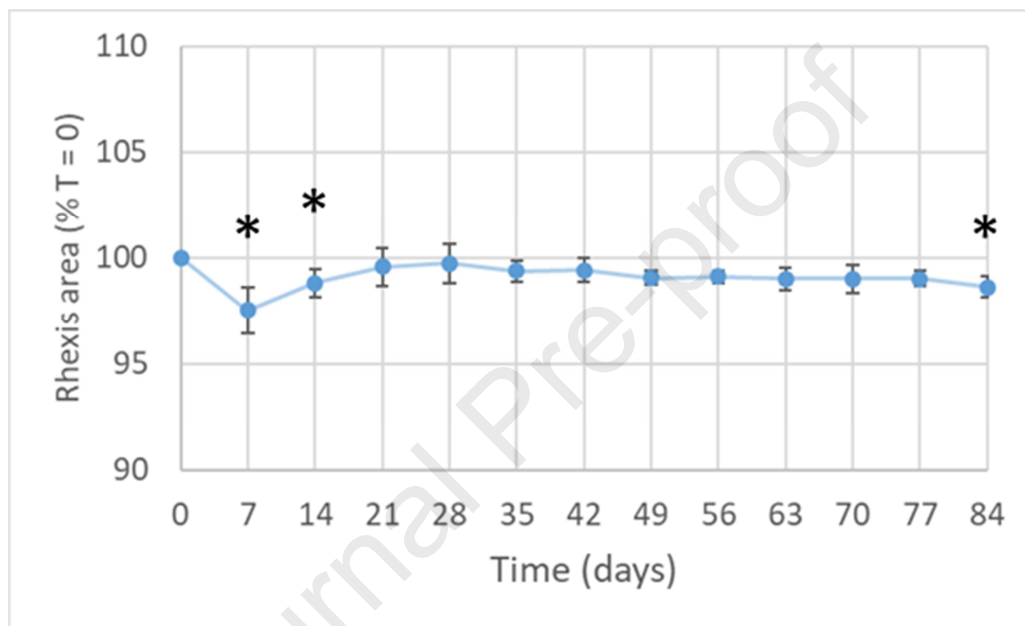


Figure 13. Quantitative assessment of the central opening, defined by the capsulorhexis over an 84-day culture period. The data are expressed as Mean \pm SEM (n=5). * indicates a significant difference from day 0 ($p \leq 0.05$; ANOVA with Dunnett's post hoc test).

4.0. Discussion

The first objective to be met was to extend the culture period of capsular bags from 28 days (Eldred et al., 2019) to 84 days. This proved successful and all cultures were maintained for the full duration. This extended culture period, demonstrated continuous change within the capsular bag. The whole bag images obtained could be analysed for a number of relevant physical measures relating to the IOL and its interaction with the capsular bag and the influence of biological events over time.

A method has been employed to provide image registration to monitor biomechanical changes over time. This method uses the haptic base as a reference point. However, using a reference point within the capsular bag is not ideal as it may not be stable throughout the culture period. To overcome this hurdle, efforts could be made to provide constant physical reference points that are independent of changes within the bag. This may be achieved through modification of the supporting ring using 3D printing to present defined features. In addition, we propose securing the ring to the culture dish rather than unattached as is the current protocol. This will allow greater consistency in image capture.

The whole bag images generated have allowed a number of quantitative measures to be obtained that provide valuable information relating to the general method and the CT LUCIA 611PY IOL per se. Assessment immediately following surgery indicated similarity within capsular bags from the same donor, but did reveal differences between donors. For instance, the size of capsular bag area differed between donors. This is likely linked to age and possibly gender (Augusteyn, 2010), however greater sample numbers are required to provide better correlation. When the IOL was introduced in to the bag, it caused a loss of circularity, such that a long axis is established due to pressure exerted by the haptics on the equatorial region of the capsular bag. This gives rise to an elliptical shaped bag with a short axis across the non-haptic regions of the bag. Interestingly the ratio of long/short axis, which provides an indication of circularity, was similar for all preparations and was independent of capsular bag area. To achieve this outcome, smaller capsular bags require greater contact with the haptic and thus the angle of contact between the haptic and capsular bag inversely correlated to capsular bag area. The angle of contact increased with culture in all cases, but the general pattern between smaller bags and greater angle of contact was maintained. This increased angle of contact may create different tensions on the capsular bag and in particular the posterior capsule, which could influence cell behaviour and the nature of PCO progression. Measurements captured in the current study relate well to capsular bag size, angle of contact and distortion findings reported by Lim et al (Lim et al., 1998). In their study, human cadaver eyes were used and subjected to cataract surgery with IOL implantation and

visualised by posterior view, however the preparations were not cultured following observation of the capsular bag with IOL implantation. The current study provides greater information than this formative work and enables changes to be monitored over time.

Once implanted in the capsular bag, and positioned, the CT LUCIA IOL 611PY appears to be relatively stable, which concurs with clinical studies (Borkenstein and Borkenstein, 2018). To assess IOL position and stability, measures between the haptics were established. The notch on the LUCIA haptic is close to the base and unlikely to deviate over time, whereas the tip of the haptic is more likely to bend applying a similar principle to a fishing rod. The distance between the corresponding reference points on each haptic were measured and did not significantly change over time, suggesting the IOL itself is relatively stable within the capsular bag.

Extending the culture period beyond 28 days showed that cells within the capsular bag remained active and changes within the bag were observed throughout. Refractive structures could be seen developing in the peripheral capsular bag, typically within the fusion footprint that resemble lens fibre cells. Moreover, individual and groups of cells that appear similar to Elschnig's pearls were detected in several preparations. In some cases these would appear and disappear over time in a similar manner to clinical observations reported by Findl et al (Findl et al., 2010). On some occasions balloon cells were observed, which appeared to be bloated nucleated cells tethered to the capsule at a point and floating in the inter capsular space in the haptic region. These also sometimes referred to clinically as bladder or Wedl cells (Pandey et al., 2004). It is often assumed by many in the clinical and research communities that Elschnig's pearls and Wedl cells originate from the lens equator (Pandey et al., 2004), but it is apparent from the current study that refractive structures can develop anywhere within the fusion footprint and indeed to some degree in regions where fusion has not taken place. These observations suggest that the graded culture regime employed can support differentiation within the capsular bag. Extending the period of culture allows these changes to be observed more readily than a shorter experimental period. It remains to be seen if further extension of the culture period can yield more pronounced Soemmering's ring and Elschnig's pearl formation attributed to Regenerative PCO.

The putative benefits of a square-edge optic barrier are a major focus in IOL development (Perez-Vives, 2018). The CT LUCIA 611PY has a step-vaulted haptic design created to provide stability and to increase contact between a 360° optic edge and the posterior capsule. In relation to the barrier effect, we should take into account the sandwich theory of PCO, which suggests that lens cells adhere to both bioactive IOL materials and the posterior capsule to increase interaction between the optic edge and capsule (Linnola, 1997). This in

turn is thought to impede cell movement on to the posterior capsule. The data from the current study established a sequence of events that better explain the role of the optic barrier in PCO management and challenge aspects of the sandwich theory. The first interesting feature is that the optic edge provides limited impedance to the first wave of growth across the PC, behind the IOL. This observation tallies with other square-edge commercial IOLs tested in the model (Eldred et al 2014; Eldred et al 2019). It is worth noting that this initial growth beyond the optic edge takes place prior to fusion of the anterior and posterior capsules taking hold. Once the anterior and posterior capsules connect – fusion footprint – the barrier effect is likely to be more pronounced and limit further cell movement on to the posterior capsule. There is clear evidence at end-point that the CT LUCIA 611PY IOL had interacted with the capsule and formed a barrier. Indeed this appeared to be 360° in most, if not all preparations. The restriction in cell movement resulting from the strong interaction of the CT LUCIA 611PY optic edge and the capsule is likely to limit cell populations and PCO progression within the visual axis. It is reasonable to propose that this IOL/capsule interaction becomes more pronounced as the fusion footprint develops and in doing so compartmentalises the posterior capsule in to two populations i.e. one group of cells beneath the optic and a second peripheral to the optic edge. The sandwich theory suggests cell interaction with the IOL, with fibronectin serving as biological glue, plays an important role in establishing the barrier effect (Linnola et al., 2000a, b). However, removal of the IOL from the bag at endpoint was met with little resistance and few cells populated the IOL surfaces throughout culture. It is therefore likely that cell/IOL interaction as described in the sandwich theory is not required to establish optic edge capsule interaction and provide a barrier effect. Overall it would appear that the optic barrier is unlikely to prevent the initial movement of cells on to the central posterior capsule, but over time the optical barrier becomes more effective as the fusion footprint develops, which will prevent/restrict further encroachment of cells behind the IOL and limit PCO progression.

An interesting observation within the study was the initial reduction in capsulorhexis size followed by an increase over time. The pattern observed may link to different phases of stability within the bag. Following surgery the anterior and posterior capsules are not connected. Cell recolonisation of the anterior capsule adjacent to the rhexis may cause the contraction of the rhexis. Once the fusion footprint forms in the peripheral bag, this may pull the capsule and open up the rhexis i.e. increase size.

In summary, we have been able to extend the culture period of the capsular bag model to a three-month duration, which has allowed more clinical features of PCO to be observed. Importantly, we have been able to establish methods to quantitative outputs/measures

relating to IOL stability and the capsular bag. The capsular bag model allows better understanding of IOL characteristics in a clinically relevant biological system both immediately following surgery and in the context of post-surgical wound-healing that leads to PCO. The model consequently provides an excellent platform to facilitate IOL development and permits comparison of IOL stability and anti-PCO performance to be assessed.

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5.0. Acknowledgements

The authors thank The Humane Research Trust for providing the infrastructure and facilities that allowed this work to take place. We are also grateful to NHSBT for the provision of donor tissue and our colleagues for their views and evaluation of the manuscript.

6.0. Funding sources

Carl Zeiss Meditec AG and the Humane Research Trust

7.0. References

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Highlights

- The human graded culture capsular bag model can be studied for three-months
- The model presents features of both fibrotic and regenerative PCO
- Biomechanical interaction can be measured and compared over time using the model
- The CT LUCIA 611PY IOL is stable within the bag and has a strong optic barrier effect
- Optic barrier results from anterior/posterior capsule fusion in the peripheral bag

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