A Fully Human In Vitro Capsular Bag Model to Permit Intraocular Lens Evaluation

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PURPOSE. To establish a fully human in vitro culture model with which to test the putative effects of intraocular lens (IOL) designs in preventing posterior capsule opacification (PCO) after cataract surgery.

METHODS. A sham cataract operation was performed to prepare human capsular bags from donor lenses. In one capsular bag of a donor pair, an intraocular lens (PMMA round-edge IOL or acrylic IOL) was implanted while the other capsular bag remained aphakic. Bags were transferred to a Petri dish and secured anterior-face down using entomological pins. Capsular bags were maintained in Eagle’s minimum essential medium supplemented with 2% human serum and 10 ng/mL TGF-β to drive growth and matrix contraction.

RESULTS. In the absence of an IOL, cells appeared within the central posterior capsule at 4.38 ± 0.26 days, whereas in the presence of a PMMA round-edge IOL or an acrylic IOL they appeared at 8 ± 0.41 days and 11 ± 0.7 days, respectively. Immunocytochemical analysis showed an accumulation of cells at the edge of the acrylic IOL and a less evident accumulation with the PMMA round-edge IOL. Moreover, matrix contraction was more prominent in the absence of an IOL but was still apparent, to a lesser degree, in the presence of a PMMA round-edge IOL. The acrylic IOL greatly suppressed matrix contraction.

CONCLUSIONS. The authors have developed a fully human in vitro capsular bag system that relates well to clinical observations and permits the testing of novel intraocular lenses. (Invest Ophthalmol Vis Sci. 2012;53:23–29) DOI:10.1167/iovs.11-8851

Cataract is a consequence of the aging of the lens and is the major priority in the global initiative to eliminate avoidable blindness by 2020. At present, the only means of treating cataract is by surgical intervention, which initially restores high visual quality. Unfortunately, posterior capsule opacification (PCO) is a common complication of cataract surgery and develops in a significant proportion of patients to such an extent that a secondary loss of vision occurs.

Modern cataract surgery generates a capsular bag, which consists of a portion of the anterior capsule and the entire posterior capsule. The bag remains in situ, partitions the aqueous and vitreous humors, and, in most cases, houses an IOL. The production of a capsular bag after surgery permits free passage of light along the visual axis through the transparent IOL and thin acellular posterior capsule. However, on the remaining anterior capsule, lens epithelial cells stubbornly reside despite their having endured the rigors of surgical trauma. This resilient group of cells then begins to recolonize the denuded regions of the anterior capsule, encroach onto the IOL surface, occupy regions of the outer anterior capsule, and, most important, start to colonize the previously cell-free posterior capsule. Cells continue to divide and cover the posterior capsule and can ultimately encroach on the visual axis. A thin layer of cells is insufficient to affect the light path, but subsequent changes to the matrix and cell organization can give rise to light scatter. If these changes are sufficiently severe, vision is then seriously impaired and corrective laser surgery is required.

A number of factors can increase the incidence of PCO, such as age, inflammatory conditions, and surgery. Recent studies have shown that PCO rates can be diminished by improved IOL design. These designs are manufactured using a range of materials and are likely to influence the progression of PCO because of the physical association with the capsule, creating a barrier effect. However, there remains a significant need to lower the need for capsulotomies, which place a strain on health care resources and cause inconvenience for patients. However, it should not be assumed that a person who does not undergo corrective laser surgery has optimal vision. Laser surgery is used when visual loss significantly affects the patient’s quality of life. Using laser capsulotomy rates as a barometer for PCO is a useful tool, but a number of patients who do not undergo this secondary surgery are still likely to have diminished visual quality. Sundelin and Sjorstrand examined this group of patients and found that a significant proportion would benefit from laser capsulotomy. Other very important considerations are that in developing countries laser treatment may be difficult to access and that in pediatric surgery laser treatment is often not possible without general anesthesia. Therefore, despite the progress made with IOLs, the problem of PCO after cataract surgery is far from resolved.

During IOL surgery the patient receives an implant that will help restore refractive power, but corrective lenses are often still needed because these IOLs are relatively inflexible and have limited accommodation. Despite the general interest in accommodative IOLs, the aberrant proliferation of lens epithelial cells and the resultant increase in light-scattering modifications and capsule fibrosis may become limiting factors. The new focusable IOL technology will reduce the association of the IOL with the lens capsule because of the dynamic nature of this technology; consequently, the barrier effect will be minimized.

Until a mechanism is developed to prevent the cellular changes that give rise to PCO, modification of existing IOL designs will continue to be an important line of investigation.

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At present, IOLs are largely tested on rabbits to determine their relative benefits. This choice is made because of similar dimensions of the rabbit lens and the human lens. However, the rabbit lens exhibits a far more severe response to injury than the human lens.13 We believe that development of our human in vitro capsular bag model will serve as a valuable tool with which to assess new IOL designs that will reduce the use of animals for this purpose. Therefore, we aimed to establish a fully human in vitro culture model to test the putative effects of IOL designs in preventing PCO after cataract surgery.

**METHODS**

**Assessment of Growth Potential Using Human Lens Epithelial Explants**

An outline of the procedure used is provided in Figure 1. In essence, human donor lenses were obtained with national research ethics committee approval and were used in accordance with the tenets of the Declaration of Helsinki. Lenses were dissected such that the anterior capsule and the associated epithelial cells were isolated from the fiber cells that form the bulk of the lens. The preparations were then cultured in serum-free Eagle’s minimum essential medium (EMEM) until cells had recolonized denuded regions of the capsule. Once a sheet of cells had been established, the epithelium was dissected into quarters, transferred to separate dishes, and maintained in different levels of human serum (Sigma, Poole, Dorset, UK)—supplemented EMEM for 7 days. At the end point, cells were evaluated using BrdU labeling and detection.

**BrdU Protocol**

A commercial BrdU kit (Promega, Madison, WI) was used to evaluate cell proliferation. In brief, BrdU was added to the culture medium at a concentration of 10 μM for the final 2 hours of the experiment, followed by washing three times in phosphate-buffered saline (PBS). Preparations were then fixed for 30 minutes with cold ethanol (70% ethanol in glycerol buffer, 5 μM, 20°C). After this, preparations were washed three times for 5 minutes each in PBS before the application of hydrogen chloride (2 N), incubation at 37°C for 20 minutes, and subsequent washing in PBS. Monoclonal anti-BrdU (1:250) was applied for 30 minutes, followed by three washes in PBS. Preparations were covered with alkaline phosphatase-labeled sheep anti–mouse antibody, and BrdU was visualized with nitroblue tetrazolium and X-phosphate. After 10 minutes, the reaction was stopped by washing the preparations in double-distilled water. Counterstaining was performed for 1 minute with nuclear fast red. Images were then observed using a standard light microscope (Nikon, Tokyo, Japan).

**Capsular Bag Preparation**

As previously described, modern cataract surgery involves the generation of a lens capsularg bag. It is this procedure that has been transferred from the operating theater to the laboratory. An adaptation of the model previously described by Liu et al.14 was used such that sham cataract surgery was performed in a laminar flow hood on eight whole donor eyes (mean age, 57.1 ± 5.1 years). Donor eyes were obtained within 48 hours of death with national research ethics committee approval and were used in accordance with the tenets of the Declaration of Helsinki. The procedure involves washing the lens briefly with EMEM before creating a small rhexis in the anterior surface of the lens capsule and removing the central fibrous mass from donor globes. The product of this operation is a capsular bag; when required, it can house an IOL. The capsular bag containing an IOL can then be removed from the eye after careful separation of the zonules, transferred to a tissue culture dish, and placed anterior-face down. This provides better physical interaction between the IOL and the capsule, which is more...
reflective of in vivo forces. The bag is subsequently secured to the dish using entomological pins (by penetration of the anterior and posterior capsule) and maintained in EMEM supplemented with 2% human serum, 10 ng/mL TGF-β2, and 50 ng/mL gentamicin (Sigma) for 28 days. Coverage of the previously cell-free posterior capsule is captured using low-power phase microscopy.

Immunocytochemistry

All reagents were from Sigma unless otherwise stated. Washes were for 3-15 minutes in PBS/BSA/nonionic detergent (Igepal; Sigma) (0.02% and 0.05%, respectively). Capsular bag preparations were fixed for 30 minutes in 4% formaldehyde in PBS and permeabilized in PBS containing 0.5% Triton X-100, also for 30 minutes Nonspecific sites were blocked with normal goat serum (1:50 in 1% BSA/PBS). Antivimentin (clone V9; Sigma) was diluted 1:100 and applied for 60 minutes at 35°C, followed by washing. Vimentin was visualized with Alexa Fluor 488–conjugated secondary antibodies (Molecular Probes, Leiden, Netherlands). The F-actin cytoskeleton and chromatin were stained with Texas Red-X-phalloidin (Molecular Probes) and DAPI for 10 minutes at room temperature. The stained preparations were again washed extensively, floated onto microscope slides, and placed in mounting medium (Hydromount; National Diagnostics, Hull, UK). Images were viewed with a Zeiss epifluorescence microscope and Zeiss software (Axiovision).

Intraocular Lens Assessment

We tested an Alcon AcrySof MA60BM IOL (Alcon Laboratories, Inc., Fort Worth, TX) with a 6-mm optic and 13-mm haptic diameter and a Rayner PMMA 702U IOL (Rayner Intraocular Lenses Limited, East Sussex, UK) with a 7-mm optic and 13.5-mm haptic diameter. Both, in relation to PCO, have a large body of associated clinical data. The PMMA IOL is known as a round-edge type, such that the edge of the IOL optic is curved rather than square. Clinically, it is well known that square-edge IOLs, such as the AcrySof IOL, have a lower PCO incidence. A critical aspect in our method is that when the capsular bags are secured to the dish using entomological pins, the anterior surface is adjacent to the dish and the posterior surface is uppermost (Fig. 2). This allows the IOL to engage the capsule in a similar manner in vivo. In the partner capsular bag, no IOL was implanted. Ongoing observations of capsular bags were made using phase-microscopy and endpoint evaluation using immunocytochemistry with fluorescence microscopy.

RESULTS

Establishing an Appropriate Human Serum Concentration

Our first objective was to identify what level of human serum was required to adequately drive the growth of lens epithelial cells. Cultures maintained in nonsupplemented medium did not demonstrate cell migration onto the tissue culture dish (Fig. 3A). At low serum concentrations (2%), there was some evidence of growth, but peak response was observed with the addition of 2% human serum (Fig. 3B). Moreover, analysis of the cells growing from the epithelial preparations using the BrdU labeling assay confirmed high rates of cell division under 2% human serum conditions (Fig. 3C). Having established that a 2% human serum supplement is optimal, we then began testing IOLs in the fully human capsular bag system.

Capsular Bag Model

In addition to using 2% human serum, capsular bag cultures were supplemented with 10 ng/mL human recombinant TGF-β2.
$\beta_2$; this combination is likely to arise in the local lens environment after surgery.\textsuperscript{2–4} Serum drives growth,\textsuperscript{15} whereas TGF-$\beta_2$ induces cells to contract the collagen lens capsule causing wrinkling to occur, resulting in light scatter.\textsuperscript{16,17}

Our first key observation was the point at which cells could be seen growing on the central posterior capsule (i.e., within the rhexis margin). This was particularly important because it corresponded with the visual axis; thus, changes that occur here can affect visual quality. When capsular bags were maintained without an IOL, cells typically were observed at the day 4 time point (Fig. 4). The presence of an IOL affected at which point cells growing within the central posterior capsule was determined. This observation was made at day 8 ± 0.41 in the presence of a Rayner PMMA round-edge IOL and at day 11 ± 0.7 in the presence of an Alcon AcrySof acrylic IOL (Fig. 4). This general pattern ties in well with clinical data\textsuperscript{18}; the further delay with the Alcon AcrySof acrylic IOL can be attributed to the well-described barrier effect from the interaction of the capsule and the square edge of this IOL.\textsuperscript{10} At day 28 (end point), low-power images revealed that the percentage coverage of the posterior capsule differed between the groups (see Fig. 6). Without an IOL, or with a Rayner PMMA round-edge, the entire posterior capsule became covered with cells (Fig. 5). However, the Alcon AcrySof acrylic IOL reduced growth; though some coverage of the posterior capsule was observed, it was not complete (Fig. 5). This pattern was observed in all four experiments using this IOL. In the absence of an IOL, marked wrinkling occurred. However, the presence of an IOL reduced the severity of wrinkling (Figs. 6, 7). End point analysis using immunocytochemistry further confirmed the presence of cells on the posterior capsule of capsular bags maintained without an IOL and in the presence of a Rayner PMMA round-edge (Figs. 7, 8). These data also reveal a number of interesting findings relating to cells within capsular bags maintained with Alcon AcrySof acrylic IOL. In the example shown in Figure 7, negligible coverage of the central posterior capsule had occurred. Moreover, the images reveal an accumulation of cells in the region adjacent to the IOL edge (Fig. 7, asterisk). This provides evidence that the barrier effect can be mimicked in our in vitro system and is likely to explain the delay in cell growth presented in Figure 4. In addition, the cells that manage to breach the barrier created by the square-edge appear to reside on the posterior capsule at lower density than observed with capsular bags maintained in the presence of a Rayner PMMA round-edge (Fig. 8). This could also explain why the degree of contraction/wrinkling was markedly reduced. In addition, cell growth on the outside of the anterior capsule was observed in all preparations (Fig. 9). Changes to these cells can lead to anterior capsule fibrosis, which can affect IOL positioning and reduce visual quality. The degree of this growth was similar in all groups.

**DISCUSSION**

The implantation of an IOL not only restores refractive power, it plays a key role in the prevention or retardation of PCO formation.\textsuperscript{4,18} Capsular bag models have been used to study PCO, and a variety of methods have been used to maintain their shape.\textsuperscript{14,19–23} With respect to testing IOLs using such systems, the body of literature is limited.\textsuperscript{14,19,22,24} Liu et al.\textsuperscript{14} initially demonstrated that an IOL could impede growth to some degree using the human capsular bag system developed in Norwich. In the present study, we made a modification in the protocol such that the capsular bag containing the IOL was inverted, resulting in the anterior capsule being closest to the plastic dish. This engages the IOL with the posterior capsule and allows the barrier effect to be tested. Moreover, coverage of the posterior capsule can be observed using standard microscopy. Despite being anterior-face down, the capsular bags still
exhibited general growth characteristics similar to bags cultured anterior-face up, suggesting that adequate supplies of survival and growth factors are available to the cells. This may indicate that a gap between the anterior capsule and the dish is in place to allow this surface to be bathed or that there is an exchange between the inner bag and the posterior capsule. It is possible that pinning directly through the capsule can distort the capsular bag and, therefore, prevent the IOL locating in its natural position; however, using pins to secure the capsular bag has provided results that reflect clinical features of PCO, thus demonstrating the value of this approach. Another variant of the capsular bag system that was recently published by Cleary et al.19 retains the ciliary body, which is pinned to a silicon ring. Using this method,
the capsular bag is effectively suspended and can house an IOL, which can also be observed. This approach requires greater volumes of media than the system presented here and will always be at greater risk for infection because of the remaining soft tissue. The suspended model does, however, have the advantage that the capsular bag is not penetrated. Both the suspended system and the inverted Norwich model provide valuable methods that can be used to assess the properties of IOLs. A particular feature of the present study is that it is a fully human system using human serum and human recombinant TGF-β to drive growth and matrix contraction. It will, therefore, be interesting to apply the same strategy to the suspended bag model and to compare observed outcomes with the inverted Norwich model.

In the present study, we evaluated an AcrySof square-edge IOL, which significantly delayed growth on the posterior capsule compared with cells growing on capsular bags maintained without an IOL. The time that elapsed before growth was observed on the central posterior capsule was significantly delayed with the AcrySof lens compared with a Rayner PMMA round-edge IOL. It has long been established that a square-edge IOL can delay PCO formation in vivo; however, Nd:YAG capsulotomy is still required in a significant number of cases. The material of an IOL could potentially play a role in the incidence of PCO, but reports suggest the greatest factor is whether the IOL edge is curved or sharp.10 For example, it is reported that a square-edge IOL made from PMMA has the same physical properties as a square-edge lens made from acrylic and that both impede cell growth.10 Our model clearly shows that any form of IOL has benefits compared with no IOL, but a clear difference between the square-edge and the round-edge lens is evident. End point evaluation using immunocytochemistry demonstrates that cells accumulate against the IOL edge, thus demonstrating that the barrier effect can be tested in our system. In PMMA round-edge IOLs, this phenomenon was not observed.

Another feature that was observed with both IOLs was a reduction in matrix contraction in capsular bags maintained without an IOL. However, contraction was still observed in the PMMA IOL–maintained capsular bags but was limited in the AcrySof IOL group. In all cases, the rhexis sat completely on the IOL, which clinically is linked to reduced PCO rates.25 Moreover, the capsular bag is inverted to allow greater interaction between the IOL and the adjacent capsule. Therefore, the lenses are tested with the highest likelihood of success. In future studies it will be of value to test surgical imperfections, such as a rhexis, which overhangs the IOL body, and incomplete removal of fiber material. Such factors could influence the physical properties of an IOL and could have a marked effect on their ability to suppress growth and matrix contraction, consequently reducing the effect of PCO.

In summary, we have developed a fully human in vitro capsular bag system that relates well to clinical observations and permits the testing of novel IOLs in the future.
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References