Lens Regeneration in Juvenile and Adult Rabbits
Measured by Image Analysis

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Secondary cataract growth commonly occurs after extracapsular cataract extraction. The proliferation of this regrowth occurs at rates related to many factors. In this study, the authors analyzed the amount of lens regeneration after endocapsular lens extraction that leaves the anterior and posterior capsules relatively intact. The analysis was performed in New Zealand albino rabbits with the aid of image analysis measurements in young and adult animals. The effect of low vacuum suction of the anterior capsule on the growth was determined. Lens regeneration was used as a measure of the growth potential of the leftover epithelial cells in the capsule bag. The results showed that lens regeneration was significantly faster in younger rabbits. However, low vacuum suction had no effect on the growth rate. Potential therapeutic agents for preventing secondary cataracts may be better analyzed with image analysis processing of lens regeneration, a precise and rapid measurement technique. Invest Ophthalmol Vis Sci 33:2279–2283, 1992

Extracapsular cataract extraction with intraocular lens (IOL) implantation is the procedure of choice for the treatment of cataracts. The single most frequent cause of decreased visual acuity after this surgery is delayed opacification of the posterior capsule.1 This opacification occurs secondarily to anterior lens epithelial cell migration and myoblastic transformation, contributing to wrinkling and fibrosis of the posterior capsule and resulting in visual distortion.1,2 IOL implantation tends to delay the onset of opacification. The posterior convex IOL design with angulated haptics seems most successful at minimizing this problem.3,4

Numerous investigators have developed in vitro models for studying the proliferation of lens epithelial cells and secondary cataract.5 Recently, we reported study results on lens regeneration in New Zealand albino (NZA) rabbits,6 which may serve as a model for evaluating the proliferative potential of lens epithelial cells and studying the effects of various agents in inhibiting or preventing secondary cataract development. In the present study, we have quantified the amount of lens regeneration at various time points in NZA rabbits, in juvenile and adult animals, using image analysis measurement. We developed this model to accurately measure lens regeneration and used this as a parameter of the growth potential of the leftover epithelial cells. It has been reported that the rate of lens capsule opacification is faster in children and in younger animals.1,7,8 In addition, we have examined the effect of low vacuum suction for removing anterior lens epithelial cells at the time of surgery. Investigators have suggested that low vacuum suction may inhibit or delay the formation of secondary cataracts.9,10

Materials and Methods

Unilateral lens extraction by endocapsular phacoemulsification and irrigation/aspiration was performed on female NZA rabbits. Rabbits were divided into three groups according to age (weight) and procedure. Animals in Group 1 (n = 4) were older (1 yr) and weighed more (5 kg) than those in Groups 2 (n = 6) and 3 (n = 6), which were 3 mo old and weighed between 2 and 3 kg. An additional procedure was performed on Group 3 animals only. After phacoemulsification of the lenses of these animals, low vacuum suction was applied to the anterior capsule to remove residual lens epithelial cells.

Surgical Procedure

Pre-operatively, the eye designated for surgery was dilated with 1% cyclopentolate (Alcon, Fort Worth, TX) and 10% phenylephrine (Winthrop, New York, NY). Each rabbit was anesthetized with 5 mg/kg xylazine base (Haver, Shawnee, KS) and 50 mg/kg ketamine HCl (Aveco, Fort Dodge, IA) intramuscularly. Eyelashes were trimmed and the ocular area was disinfected with povidone iodine (Professional Disposables, Inc., Orangeburg, NY). A wire lid speculum...
was inserted to retract the lids, and a limbal incision was made at 11 o'clock with a 2.85 mm keratome. A 21 G phacoemulsification tip was inserted through the corneal wound and used to perform a 3 mm anterior capsulotomy and remove the lens nucleus. Balanced salt solution was used as the irrigant. Considerable care was taken to remove all lens cortical material by diligent irrigation and aspiration. The aspiration setting then was reduced (from 500 to 100 mmHg) to avoid inadvertent capsular tears. This low vacuum suction (polishing) of the capsule bag was performed in Group 3 animals to remove residual epithelial cells from the anterior capsule. At the end of the procedure, the corneal incision was closed with three interrupted nylon sutures (10-0).

At the end of surgery, 0.25 ml (20 mg) of gentamicin (Solo Pak, Franklin Park, IL) was injected subconjunctivally, and polymyxin B bacitracin-neomycin ointment (Pharmaderm, Melville, NY) was applied topically. Post-operatively, all surgically treated eyes were treated topically with 1% tropicamide (Alcon, Humacao, Puerto Rico) and 0.3% gentamicin (Allergan, Irvine, CA) four times per day for 7 d. Animals were treated in accordance with USDA guidelines and the ARVO Resolution on the Use of Animals in Research.

Slit-Lamp Biomicroscopy and Photography

Post-operatively, animals were followed with slit-lamp biomicroscopy and photography at regular intervals (weekly for the first month and monthly for up to 6 mo). At each time point, the lens regeneration that filled the capsular bag was photographed and measured with the pupil maximally dilated. Lens regeneration proceeds from the periphery of the capsular bag to the center, and full growth (100%) was noted when the capsular bag appeared full when viewed frontally in the maximally dilated eye (Figs. 1 and 2). This was established by observing animals at the slit-lamp and later by evaluating slit-lamp photographs at the end of the study period. Photographs were made with a Nikon (Tokyo, Japan) camera attached to a Nikon slit lamp using Kodak (Rochester, NY) Ektachrome 100 film rated at ISO 100/21. The film lots were not controlled throughout the study period. Previous work done by one of the authors indicates that film lot variation is less than 1/6 of an f stop, which is less than the system noise resulting from eye movement and reflection differences.11

Image Analysis

The slit-lamp photographs were evaluated with the aid of a Macintosh II CX computer with 32-bit Color Quickdraw (Apple Computer, Cupertino, CA). A CCD camera (Sony [Tokyo, Japan] XC-77) with a 55 mm Micro-Nikkor f/2.8 (Nikon) was used to image the photographic slides. The images were digitally captured and stored on the computer. Digitization is the process of taking the analog image and creating discrete elements from that image, referred to as pixels (picture elements). All image analysis was conducted with two programs (Enhance v1.0.1 and Image v1.22y, Micro Frontier, Inc., Des Moines, IA).
Each slide was placed on a view box using daylight balanced fluorescent lighting. Camera lens and shield were shielded from stray ambient light during the imaging process. As each slide was viewed with the CCD camera, the image was transmitted into the computer through a Scion FG-2 "frame grabber" board (Image Systems Technology, Walkersville, MD). The image was viewed and stored on the computer using the Enhance software. An entire series for each animal eye was imaged during a single session to minimize artifacts, such as lighting variation, introduced during the image acquisition phase.

The image on the slide was processed into 348,160 pixels, creating a 680 × 512 rectangle of pixels. The earliest image in the series for each animal was designated as the baseline image. All other images of the eye for that animal were compared to this baseline image.

All images were geometrically aligned with the baseline image by the computer. Geometric alignment involved image rotation, size scaling, and translational (lateral) movements. Image alignment was conducted using the split screen protocols of the Enhance program, based on manually identifying common landmarks between the images. Once the landmarks were indicated, the program automatically performed the geometric alignment.

After image alignment, the measurement of lens regrowth could be calculated with a separate computer program (Image v. 1.22y). Lens regrowth of each eye was measured at each time frame with the aid of

![Fig. 3. Inverted analog image of the same rabbit eye, at the same time point pictured in Figure 1, showing tracing done by computer with drawing tool to measure area of lens regrowth. The anterior capsulotomy area is not included in the digitized image.](image)

the computer. The region of lens-regrowth in each image was traced with a program drawing tool (Figs. 3 and 4). Next, the computer calculated the area within the boundaries of the tracing. Occasionally, two or three separate areas within an image were combined into a single region of regrowth for the image (the total area of lens regrowth for that image). The computer measurements were given in arbitrary units. The units from each measurement from the different images in a sequence were followed and recorded over time. Measurement results in units then were calculated into percentages using zero as the baseline. For each rabbit, the largest or oldest growth time measurements were scaled to 100% or maximal fill (subtracting area of capsulotomy adhesion and not necessarily equivalent to 100% filling of the capsular bag; Table 1). All results were graphed individually and displayed as a mean value with standard error bars. These results then determined the amount of lens regeneration in the capsule bag. Comparisons between the groups used Student’s t-test.

**Results**

**Slit-Lamp Biomicroscopy**

In the initial post-operative period (days 1–7), no residual lens material was apparent in any of the animal eyes as observed under the slit-lamp biomicroscope. All of the eyes had mild to moderate anterior chamber inflammation that resolved by the end of the
Table 1. Image analysis lens regrowth (rabbit #7254 OD group 1)

<table>
<thead>
<tr>
<th>Weeks</th>
<th>Lens regrowth in units</th>
<th>Percent of maximal bag fill</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>3</td>
<td>6554</td>
<td>5.6</td>
</tr>
<tr>
<td>6</td>
<td>17,857</td>
<td>15.4</td>
</tr>
<tr>
<td>12</td>
<td>94,324</td>
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<tr>
<td>17</td>
<td>105,169</td>
<td>90.7</td>
</tr>
<tr>
<td>24</td>
<td>115,953</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Sample calculation of the regrowth rate observed in a 3-month-old rabbit (group 1) rabbit, as determined by digital image analysis.

first week. The eyes in Group 1 rabbits showed initial lens regeneration peripherally in the capsular bag 3–5 wk post-operatively. At 3 mo, new lens material filled approximately one half to three fourths of the capsular bag in the anterior-posterior view. At 6 mo, lens material had filled most of the capsule bag. Regenerated lens material in all of the eyes had varying translucency with some opacities and vesicles. All of the eyes showed some adhesion of the anterior and posterior capsules at the capsulotomy site only. Further lens growth appeared as a gradual thickening in an anterior-posterior direction; this was not measured.

Lens regeneration proceeded similarly in the younger animals (Groups 2 and 3), except that initial growth was 25% and first seen 2 wk after surgery. It filled one third of the capsule bag at 3 wk and filled three quarters at 2 mo. In Group 3, lens regeneration was less abundant (16% at 2 wk) than in Group 2 during the early post-operative period, but this equalized with time.

Image Analysis

Data for all three groups were calculated in a precise manner with the aid of the computer. In Groups 2 and 3 (the younger rabbits), lens regrowth filled approximately 25–38% maximal fill diameter of the capsule bag at 3 wk, whereas Group 1 (the older rabbits) had only 5% lens regrowth. By the third month, there was a marked difference in lens regrowth with 92% fill in Groups 2 and 3, compared with approximately 75% fill in Group 1. As illustrated in Figure 5, the initial lens regrowth rate in Group 1 was much slower than that of Groups 2 and 3. Comparison of the initial growth between Groups 2 and 3 in the first 2 mo showed no significant difference ($P = 0.34$). The initial regrowth rate was slightly slower in Group 3 (low vacuum) but Groups 2 and 3 equalized by 3 mo ($P = 0.86$). Within 5 mo, all of the regrowth in Group 2 and 3 animals had reached maximum fill with most regrowth attaining a plateau by months 3 to 4. The difference between Groups 2 and 3 was not significant. In all of the older animals (Group 1) maximal fill was not obtained until the sixth month (Fig. 5). The differences between Group 1 and Groups 2 and 3, in terms of attaining maximal fill, were statistically significant at the $P < 0.05$ level, using the between group t-test (Table 2).

Discussion

Previous studies have shown that lens regeneration can occur spontaneously after endocapsular phacoemulsification and irrigation/aspiration of the lens capsular contents of NZA rabbits, when the anterior and posterior capsules are left relatively intact.6,12 In the present study, we report the rate of this lens regeneration in young and old rabbits. We found that lens regeneration was significantly faster in younger animals. Lens regeneration in young animals occurred as early as 2 wk after surgery, and the capsular bag reached maximum fill with newly regenerated lens material at approximately 3 mo. In comparison, lens regeneration in adult animals was not observed until 5 wk after surgery and was still occurring as long as 6 mo later. A similar pattern occurs in humans. Posterior capsule opacification after extracapsular cataract extraction with IOL implantation occurs more frequently and at a much faster rate in children than in adults.1

Table 2. Growth table digital analysis (% maximal fill)

<table>
<thead>
<tr>
<th>Weeks</th>
<th>Group 1 % (range)</th>
<th>Group 2 % (range)</th>
<th>Group 3 % (range)</th>
<th>Groups 1 and 2 P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>(Older) 0.0</td>
<td>(Young) 0.0</td>
<td>(Young/L.V.S) 0.0</td>
<td>0.017</td>
</tr>
<tr>
<td>2</td>
<td>5.1 (2–7)</td>
<td>25.4 (10–40)</td>
<td>16.7 (7-26)</td>
<td>24.6 (17–50)</td>
</tr>
<tr>
<td>3</td>
<td>16.2 (14–18)</td>
<td>38.2 (30–55)</td>
<td>45.2 (30–75)</td>
<td>0.009</td>
</tr>
<tr>
<td>6</td>
<td>83.2 (65–85)</td>
<td>72.5 (65–80)</td>
<td>91.3 (74–100)</td>
<td>95.1 (85–100)</td>
</tr>
<tr>
<td>8</td>
<td>74.5 (60–85)</td>
<td>91.3 (74–100)</td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>

Comparison of rates of lens regrowth in 1-year-old (group 1), and 3-month-old (group 2 and group 3) rabbits.
Image processing of the photographic data proved to be a precise, rapid method of data analysis. Image processing reduced errors of image magnification and misalignment inherent in animal studies. In addition, the methodology described in this report permitted quantification of photographic documentation of lens regeneration.

In conclusion, we have demonstrated that the growth curves for lens regeneration differ with age in NZA rabbits after endcapsular phacoemulsification of the lens and irrigation/aspiration. In addition, we have described a new quantitative method for analyzing this lens regrowth using image analysis. We hope this method will serve as a useful model for evaluating potential therapeutic agents in the prevention of secondary cataracts and for conducting further research in lens regeneration.

Key words: lens regeneration, lens regrowth, secondary cataract, image analysis measurements

References