A Histologic Study of Lens Regeneration in Aphakic Rabbits

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Lens regeneration occurs in New Zealand albino rabbits after endocapsular lens extraction, which leaves the anterior and posterior lens capsules relatively intact. Slit-lamp photography, histologic studies, and lens protein analysis confirmed the differentiation of lens fibers. In the current study, we performed a sequential analysis of the regenerating rabbit lens. After endocapsular phacoemulsification and irrigation/aspiration of the lens, rabbits were sacrificed at different time points for histologic evaluation. Similarities with embryologic development of the lens were evident, although in some sections, abnormal cellular proliferation occurred. By the 6th day after surgery, a monolayer of lens epithelial cells lined both the anterior and posterior capsules. At 1 month, the posterior epithelial cells had elongated, and nuclei had migrated anteriorly. At 2 months, lens cells were differentiating at the equatorial zone with gradual elongation, anterior migration of nuclei, and eventual loss of nuclei. Invest Ophthalmol Vis Sci 31:540–547, 1990

The progressive steps in the process of spontaneous regeneration of tissue of ectodermal origin have been described well for the areas of skin epidermis and corneal epithelium.1,2 Another ectodermal derivative, the crystalline lens, was reported in 1827 to regenerate in rabbits.3 However, research in this area has progressed more slowly. Investigators have found that the lens regenerative process is dependent on an intact anterior and posterior lens capsule.4 After extraction of the lens capsular contents, regenerating lens tissue first is noted 2 weeks postoperatively, beginning in the periphery of the capsule and occurring more rapidly in younger rabbits.5

In 1842, Valentin described for the first time the regenerated rabbit lens on a microscopic level, demonstrating the presence of characteristic round or polyhedral-shaped crystalline cells.6 He suggested that regeneration takes place by effusion into the capsule of initially liquid cytoblastic masses, which subsequently develop into lens cells and fibers.

Thirty years later, in 1872, Milliot reported that lens fibers formed in the equatorial region and were aligned linearly, similarly to the lenses in embryos or very young rabbits.7 Furthermore, the regenerated lens contained the same anatomic elements present in a normal lens, ie, epithelial cells and lens fiber cells with larger than normal interfibrillar spaces.

According to Randolph (1899), Gonin noted enlargement and karyokinesis of the epithelial cells on the anterior capsule as early as 1 day after surgery, with full proliferation occurring 2 days postoperatively.7 In some cases, Gonin found that anterior capsule cells became detached and acted as centers of proliferation on the posterior capsule. In the equatorial region, at the most peripheral parts of the posterior capsule, epithelial cells were seen to extend and elongate. The lens fibers that were not removed during surgery tended to swell and become granular. From these observations, Gonin concluded that the new lens was formed by the hypertrophy of the lens fibers remaining after surgery and continued to develop at the equatorial zone.

In 1960, Stewart showed that when embryonic tissue was implanted into the capsular bag after lens evacuation, the new lens fibers were aligned in the typical concentric pattern of the mature lens.8 He demonstrated also that lens differentiation occurred at the equator.

More recently, Gwon et al showed that regenerated rabbit lenses differentiate normally at the equatorial zone and also at the anterior capsulotomy site where anterior and posterior capsules are in close approximation.9 In addition, Gwon and Enomoto verified the production of α, β, and γ crystallins in the regenerated rabbit lens with proportions similar to fetal or normal lens.10

To better understand the process of lens regeneration or healing after endocapsular lens extraction,
and to verify that the process develops from residual lens epithelial cells (rather than retained lens fibers), we examined the histologic findings in the early postoperative period during the phase of early lens regeneration in rabbits. Previous histologic work has focused on the developed regenerated lens when lens fiber differentiation is restricted to the lens equatorial zone.5,7,8

Materials and Methods

Surgical Procedure

Six young New Zealand albino rabbits, weighing approximately 2.5–3 kg, were anesthetized with approximately 5 mg/kg Xylazine (Fermenta Animal Health) and 50 mg/kg ketamine (Aveco, Fort Dodge, IA), intramuscularly. The surgical eye was dilated with 1% cyclopentolate (Alcon, Fort Worth, TX) and 2.5% phenylephrine (Winthrop, New York, NY); eyelashes were trimmed; and the ocular area was disinfected with povidone iodine. 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-gauge phacoemulsification tip was inserted through the corneal wound and used to perform the anterior capsulotomy and removal of the lens nucleus with balanced salt solution as the irrigant (Fig. 1A). Considerable care was taken to remove all lens cortical material by diligent irrigation and aspiration. At the completion 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 Polymixin B-bacitracin-neomycin ointment (Pharmaderm, Melville NY) was applied topically. Postoperatively, all surgical eyes were treated topically with 1% tropicamide (Alcon, Humacao, PR) and 0.3% Gentamicin (Allergan, Irvine, CA), four times daily for 7 days. All experimental

![Fig. 1. (A) Schematic diagram of endocapsular phacoemulsification technique. (B–D) Light micrographs of epithelial cells in capsular bag, 1 day postoperatively. (Anterior lens surface, right; posterior, left.) (B) Anterior epithelial cells are multilayered in some areas (×60). (C) Single layer of flat cells with elongated nuclei migrate along the posterior capsule (×60). (D) Cellular proliferation in equatorial region (×10). (Hematoxylin and eosin)
animals were treated in accordance with USDA guidelines and the ARVO Resolution on the Use of Animals in Research.

**Histologic Procedure**

Slit-lamp biomicroscopy and photography were performed periodically and rabbits were sacrificed at various time periods for histologic examination. For each rabbit, both eyes and their associated structures were removed and preserved in 10% neutral buffered formalin. After fixation, the globe was sectioned through the cornea, pupil, and optic nerve with the lens in situ. Tissues were processed in an automatic tissue processor in a 14-h processing cycle which included dehydration in reagent grade alcohol, clearing in xylene, and infiltration with paraffin. Paraffin-embedded tissues were sectioned at 5 μm and stained with hematoxylin and eosin.

**Results**

**Day 1**

Postoperatively, the corneas of the rabbits were relatively clear, with little or no edema around the corneal incision site. The anterior chamber contained moderate to heavy amounts of fibrin.

Histologically, a single monolayer of epithelial cells lined the anterior capsule, with some multilayering and some gaps where cells had most likely been lost at the time of surgery (Fig. 1B). Migrating cells, as indicated by their long flat appearance, began to appear along the posterior capsule (Fig. 1C). Cells had begun proliferating in the equatorial zone, and some eosinophilic debris was seen (Fig. 1D).

**Day 4**

The corneas were clear and nonedematous, and the fibrin reaction in the anterior chamber was resolving by the 4th day after surgery.

Epithelial cells had proliferated along the posterior capsule and were in contact with a single layer of anterior epithelial cells (Fig. 2A). Multiple vacuoles were seen in the epithelial cell layers (Fig. 2A). At the equator, among some debris and scattered neutrophils, lens fibers had begun differentiating, with anterior displacement of cell nuclei (Figs. 2B, C). Some of the differentiated fibers, however, may have been material left in the capsule bag at the time of surgery.

**Day 6**

Corneas, anterior chambers, and lens capsules were clear by the 6th day.

Histologically, a single layer of epithelial cells, elongating in an anterior–posterior direction with anterior displacement of their nuclei, lined the posterior capsule (Fig. 3A). In general, the capsules were linear and regular (Figs. 3A, B). Toward the central region of the lenses, the capsules were wrinkled and undu-
Fig. 3. Light micrographs, 6 days postoperatively. (Anterior lens surface, right; posterior, left.) (A) Posterior capsule cells elongated with anterior displacement of nuclei (×10). (B) Posterior capsule cells elongated with anterior displacement of nuclei (×60). (C) Irregular cellular proliferation associated with capsular wrinkling (×10). (D) Anterior capsule rolled outward at site of surgical incision (×10). (Hematoxylin and eosin)

lating. Here, in the central region, multiple stellate cells with large vacuoles were seen in a hyperproliferative state (Fig. 3C). Similarly, a proliferation of cells with less definitive orientation was seen at the site of the anterior capsulotomy, where the capsules were less taut (Fig. 3D).

**Day 7**

Under slit-lamp examination, new lens growth was seen in the periphery of the capsular bag, and the anterior capsulotomy appeared to be sealed by fibrin in some instances (Fig. 4A).

Posterior lens cells continued to elongate in an anterior-posterior direction, and nuclei were displaced anteriorly (Fig. 4B). Cells in the equatorial zone had begun to differentiate, and nuclei had begun to migrate anteriorly. Eosinophilic, partially disorganized, lens material filled the space between anterior and posterior epithelial cells (Fig. 4C). In some cases development progressed at different rates, as in the case pictured (Figs. 4C, D), where development in the equatorial region (Fig. 4C) was more advanced than in the central lens region (Fig. 4D).

**Month 1**

By 1 month, slit-lamp examination revealed that approximately 50% of the capsular bag was filled with lens regrowth (Fig. 5A).

Histologically, the differentiating fibers from the posterior cells had elongated considerably and the nuclei had migrated anteriorly, forming a horizontal band across the posterior cortical region (Fig. 5B). In the equatorial region, normal fiber differentiation continued, with elongation of cells and anterior displacement of nuclei along the posterior capsule (Fig. 5C).

A single layer of cuboidal epithelial cells and vacuoles lined the anterior capsule; the lens cortex be-
neath was vacuolated and in some areas had separated from the anterior epithelial cells (Fig. 5B).

**Month 2**

Between the 2nd and 3rd months, lens regrowth filled the capsular bag, except in the area of the original anterior capsulotomy (Fig. 6A).

At this time, differentiation was occurring primarily in the equatorial region (Fig. 6B). The bulk of the lens material consisted of an eosinophilic amorphous substance with multiple vacuoles. In some areas, with fewer vacuoles, the lens material was uniformly arranged (Fig. 6C). In other areas, with larger vacuoles, the anterior lens cortex had separated from the epithelium (Fig. 6D).

**Discussion**

Lens fiber differentiation in the embryo has been shown to involve loss of mitotic activity, marked cellular elongation, intensive synthesis of lens specific proteins called crystallins, and loss of the cell nucleus. In this microscopic study of the regenerating rabbit lens, we have demonstrated the processes lead-
Fig. 5. Light micrographs of lens fiber differentiation, 1 month postoperatively. (Anterior lens surface, right; posterior, left) (A) Regenerated lens material at 1 month. Approximately 50% of the capsular bag is filled with new lens growth. (B) Multiple vacuoles in anterior cortex. Lens fiber differentiation proceeding from posterior capsule (X10). (C) Lens fiber differentiation in equatorial region (X10). (Hematoxylin and eosin)

followed by elongation of the posterior epithelial cells and migration of the nuclei anteriorly (1 month). During the 2nd month, lens differentiation occurs at the equatorial zone, with gradual elongation of cells, anterior migration of nuclei, and eventual loss of the nuclei. A description of the crystalline changes during lens regeneration is presented in a previous publication.9

The ability to study the differentiation of lens fiber cells in vitro has greatly enhanced knowledge of the biochemical influences on cellular differentiation. Following the suggestion of Coulombre and Coulombre that a substance from the posterior segment induces lens fiber differentiation,12 various investigators have isolated this type of factor in the retina and vitreous. Chamberlain and McAvoy have identified a factor from the neural retina that appears to be fibroblast growth factor, capable of inducing lens fiber differentiation.13 Similarly, Beebe and associates have isolated a factor, lentropin, from the vitreous; it is related to insulinlike growth factors and is capable of inducing lens epithelial cells to differentiate into fibers.14

While in vitro studies enable us to isolate biochemical factors, in vivo studies may increase understanding of the morphologic factors involved in lens regrowth. In our study, after evacuation of the lens, the empty capsular bag became folded as a result of loss of elasticity. The area of undulating or wrinkled capsule was associated with abnormal cellular proliferation. This finding suggests that the mechanical forces exerted on the capsule bag may play an important role in lens fiber differentiation. This is consistent with the work of Coulombre and Coulombre, who showed that lens fiber differentiation occurred when lens epithelial cells attached to capsules were implanted in chick embryos.15 The new lens fiber “size, shape, and orientation is remarkably better when the implant is flat.”15 This also supports the hypothesis proposed by Bito et al, that “physical forces exerted on the lens (such as those due to the varying tension of the suspensory ligaments) and the resulting wear and tear on the epithelium may be casually related to the rate of cell proliferation and the distribution of dividing cells in this tissue.”16

In summary, the regenerative process in the New Zealand albino rabbit after endocapsular lens extraction appears to follow the stages seen in the embryonic development of the lens. In the future, in the immediate postoperative period, it is possible that capsule tension may be maintained with pharmacologic agents, and that growth factors may be added to improve the quality of the regenerating lens.
Fig. 6. Light micrographs of regenerated lens, 2 months postoperatively. (Anterior lens surface, right; posterior, left) (A) Regenerated lens material at 2 months. New lens growth fills capsular bag except at the anterior capsulotomy site. Arrow points to the anterior capsulotomy site. (B) Lens fiber differentiation in the equatorial region (x10). (C) Single layer of more normal anterior epithelial cells is associated with more uniform arrangement of lens cortex (x10). (D) Anterior cortex consists of amorphous eosinophilic material with vacuoles (x10). (Hematoxylin and eosin)

Key words: lens regeneration, lens fiber differentiation, lens histology, endocapsular lens extraction, lens capsule

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