Chapter 5

TREATMENT OF MYOPIA

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This work has been done in collaboration with Joyce Huynh at Caltech, Dr. Marco Coassin and Dr. Keith Duncan at University of California at San Francisco, and Dr. Sally McFadden at University of Newcastle. As a first year graduate student, Joyce has assisted with in vitro expansion tests and with in vivo experiments on rabbits. Marco provided surgical expertise in both rabbits and guinea pigs. Keith has assisted with procurement of rabbits necessary for this work as well as with conduction of biocompatibility studies. Sally provided the guinea pig animal model of myopia and conducted expert measurements and analysis of ocular parameters. The data related to these guinea pig studies are a subset of results from a report provided by Dr. McFadden.
5.1 Introduction

In degenerative myopia, the reduction of collagen fibril diameter, enhanced turnover of scleral collagen, and alteration of scleral glycosaminoglycans results in mechanical changes to the sclera. Progressive elongation of the eye in degenerative myopia is thought to be the result of 1) the tissue being inherently weak, 2) the sclera continuously being remodeled, or 3) a combination of these. From studies of human donor tissue, high myopia is associated with weakening and thinning of the sclera, a reduction in matrix material, and reduction in collagen fibril diameter. While refractive errors induced by progressive myopia are readily corrected by spectacles, contact lenses, corneal refractive surgery, or intraocular lenses, these modalities do not prevent visual loss induced by stretching of chorioretinal tissues. Current means to treat choroidal neovascularization in degenerative myopia, such as photodynamic therapy, are minimally effective, and studies have only recently begun to test injections of anti-angiogenic drugs such as bevacizumab (Avastin®), or Lucentis®. Various attempts have been made to treat expansion of the eye due to myopia, including the use of scleroplasty, scleral reinforcement, and even an attempt to polymerize foam around the eye. Largely because these modalities remain unproven in well-controlled clinical trials, none have been widely adopted to manage patients with degenerative myopia. Current therapies are essentially palliative, attempting to mitigate visual loss in this condition.

Crosslinking of scleral components has the potential to halt progression of degenerative myopia because it addresses both of the underlying causes that are currently hypothesized: crosslinking increases tissue strength and hinders tissue remodeling. As mentioned in
Chapters 1 and 3, Wollensak and Spoerl have reported the use of collagen cross-linking agents, including glutaraldehyde, glyceraldehyde, and riboflavin-UVA treatment, to strengthen both human and porcine sclera in vitro. Glutaraldehyde and glyceraldehyde would be difficult to spatially control, and unwanted crosslinking of collagen in vascular and neural structures might have particularly untoward effects. Use of light-activated riboflavin would seem preferable in this regard; however, when testing on a rabbit model, “serious side-effects were found in the entire posterior globe with almost complete loss of the photoreceptors, the outer nuclear layer and the retinal pigment epithelium (RPE).”

While crosslinking near the posterior pole would increase scleral modulus and potentially arrest myopic progression, there remains a need for a non-toxic crosslinking agent that could be activated using short exposure to a less-toxic light source. Our research in Chapters 3 and 4 indicates that the visible-light-activated co-initiator system of Eosin Y (EY) and triethanolamine (TEOA) has the potential to fill this need.

For transition of this treatment from the lab to clinical practice, biocompatibility and efficacy must be proven in an animal model of myopia. Current state-of-the-art animal models to study the etiology of myopia rely on 1) visual form deprivation and 2) the eye’s tendency to correct refractive errors toward emmetropia. During development, eyes tend to grow excessively upon removal of spatial vision. Form-deprivation models use this response to induce myopia either by placing semitransparent occluders over the eye, or by suturing the eyelid shut. The second animal model makes use of emmetropization of the eye, which is the process by which eyes change to focus images on the retina. When minus
or plus lenses are placed over the eye, the eye adjusts its growth to bring the image into focus.

As is observed in the human disease, form-deprivation animal models (e.g., tree shrew eyes covered with occluders for 12 days) also exhibit weakened scleral tissue (e.g., increased scleral creep rates). In these animal models, there is also a measurable change in the amount and type of collagen and proteoglycan present in the tissue, indicating abnormal remodeling of the sclera. Sustained form-deprivation in animals induces changes in collagen fibril diameter and spacing analogous to the distinctive structure observed in human donor tissue of high myopes.

Various animal models exhibit similarities to humans and each other. Eutherian mammals, such as humans, monkeys, tree shrews and guinea pigs, share the trait that “the entire sclera consists of the fibrous, type I collagen-dominated extracellular matrix”. This feature sets them apart from other vertebrates, which have an inner layer of cartilage (e.g., in chicks). Indeed, the mechanism of emmetropization during form-deprivation in eutherian mammals (remodeling of the fibrous sclera) is different from that in other vertebrates (growth of the inner cartilaginous region). Therefore, eutherian mammals provide a better model for testing treatments related to scleral remodeling for potential application in humans. In light of the fact that tree shrews and monkeys are difficult to obtain and monkeys suffer from high variability of the results, researchers have been establishing other mammalian models. Guinea pigs have recently gained acceptance due to the fact that they rapidly develop myopia, the changes are large and reproducible, and they are easy to care for. This animal provides a model that is well suited for research requiring significant numbers of
animals, and at the same time demonstrates physiological and anatomical similarities to humans.

Despite the fact that the mechanism of degenerative myopia in humans is not completely understood, the animal models of myopia do express the weakened sclera and excessive remodeling typical of the disease. As will be demonstrated in this chapter, light activation of Eosin Y/TEOA strengthens the sclera; and as discussed in Chapter 3, non-enzymatic collagen crosslinking is known to decrease enzymatic degradation. Therefore, treatment with Eosin Y/TEOA has the potential to address both putative mechanisms of degenerative myopia.

This chapter illustrates efficacy and biocompatibility of this potential treatment. Stabilization of ocular shape is demonstrated for *in vitro* and *in vivo* drug delivery to rabbit eyes followed by *in vitro* eye expansion using the intact globe method (Chapter 2). Preliminary safety studies in rabbits suggested no ill effect of the treatment. We have also conducted experiments to establish drug and light delivery protocols in guinea pigs and to assess the effect of EY/TEOA on ocular growth and form-deprivation myopia in collaboration with Sally McFadden at the University of Newcastle in Australia. The current results indicate that EY/TEOA has an ability to alter ocular parameters of guinea pig eyes without altering gross ocular function or animal behavior.
5.2 Materials and Methods

5.2.1 In vitro Treatment & In Vitro Expansion

The following procedures were used for testing the effect of in vitro treatment of eyes on preventing expansion of intact rabbit kit globes subjected to an elevated intraocular pressure.

Tissue Preparation: Eyes from 2–3 week old New Zealand White Rabbits (University of California at San Francisco) were stored in saline on ice for use within 48 hours of enucleation. Immediately before testing, the extraocular muscles, the conjunctiva, and the episcleral tissues around the eyes were carefully removed to expose the sclera.

Materials: Treatment solutions of 0.0289 mM EY and 90 mM TEOA in DPBS (henceforth called 1x EY) were prepared fresh. As discussed in Chapter 3, these solutions are activated by visible light and have peak absorption at 514 nm. The measured pH was 7.5 for the solution. Glyceraldehyde (GA) solution was prepared by mixing 2% by weight DL-Glyceraldehyde (Sigma) in distilled water. The pH was adjusted to ~7.5 with HCl and NaOH.

Eosin Procedure: Eyes were soaked for 5 min in 5 mL of treatment (1x EY) or control (DPBS) solution. The eyes were removed from the soak and excess solution was wiped from the surface using a Kimwipe. The treatment was activated by placing the eyes under one of two light sources: a high intensity mercury arc lamp equipped with a 450–550 nm bandpass filter that provided 34 mW/cm², or a panel of seven light emitting diodes (LEDs) with a spectral output at 525 ± 16 nm that provided an irradiance of 7–10 mW/cm², as
measured at the center position of the eye (Figure 5.1). With the arc lamp, the anterior hemisphere of the eye was exposed for 5 minutes and then the eye was flipped and the posterior globe was exposed for 5 minutes. With the LEDs, the entire eye was irradiated at once for 5 minutes. The eyes were placed in a rinse solution of DPBS for 30–45 min and then loaded on the expansion setup which has been described in detail previously (Chapter 2).

Glyceraldehyde Procedure: Because of its well-documented effects as a crosslinker, a comparison group was treated with 2% GA solution. To allow GA to penetrate into the cornea (for comparison to keratoconus treatments in Chapter 6), the corneal epithelium of enucleated eyes was removed by scraping with a scalpel blade. The eye was then soaked in 5 mL of 2% GA for 12 hours; when it was removed from the soak, excess solution was removed with a Kim Wipe. The eyes were rinsed in a 20 mL bath of DPBS for ~5 seconds,
and then put in a fresh 40 mL DPBS bath to rinse for 10 hours. The eyes were then loaded on the expansion setup.

As described in Chapter 2, the expansion protocol began with a 1 hour interval at an intraocular pressure (22 mmHg) close to the physiologic value, which allowed the globe to recover from shape distortion that may have occurred during handling post mortem. Then the pressure was raised and held at 85 mmHg for 24 hours. Digital photographs (2272 x 1704) were acquired every 15 min for the duration of the experiment.

5.2.2 Biocompatibility

Toxicity studies were performed at UCSF, to determine if the formulation and light exposure selected from *in vitro* studies would be suitable to use in an animal model for myopia. To test the *in vivo* response to 1x EY and light exposure, the following experiments were performed using topical application of the drug.

Procedure: Four adult New Zealand White rabbits were given general anesthesia with 1–5% inhaled isofluorane administered by mask and topical 0.5% proparacaine to the right eye (OD). The right eye of each animal was sterilized with 5% povidone-iodine (betadyne). Throughout the procedure the eye was washed with sterile ocular balanced saline solution (BSS). A 15 mm incision was made in the conjunctiva close to the limbus and another incision running anterior to posterior allowed the conjunctiva to be pulled away to expose the sclera over approximately 1 cm² area. The animal was positioned such that the exposed sclera faced upward and a drop of solution placed on it could remain in contact with the tissue for 5 minutes. Rabbits from Group 1 had 200 microliters of 1x EY
solution applied directly to the exposed sclera. Rabbits from Group 2 had 200 microliters of DPBS (control) applied directly to the exposed sclera.

After 5 minutes, the treated area was rinsed with 1–2 mL of BSS and then photoactivated by exposure to light from an LW Scientific Alpha 1501 Fiber Light Source (~34 mW/cm²) for 5 minutes.

The conjunctival incision was closed with 7-0 vicryl suture. All animals received subconjunctival injections of celestone (75–150 microliters) and cepahzolin (75–150 microliters). All animals were given injections of carprofen (5 mg/kg) and buprenorphine (0.05 mg/kg) for pain and 2–3 drops of neomycin, polymixin B sulfates, and gramicidin OD to prevent infection.

Eyes were examined for any signs of pain or inflammation such as redness of the eye, discharge, ptosis of the eyelid, blepharospasm, or photophobia once a day for 1 week then once a week for 3 additional weeks.

Histology: After 4 weeks all animals were anesthetized with 30–50 mg/kg ketamine and 5–10 mg/kg xylazine, euthanized, and the eyes were removed, fixed in 10% formalin, and processed for light microscopic examination (Eosin/hematoxylin stain).

5.2.3 In vivo Treatment & In Vitro Expansion

The following experiments used in vivo treatment of the eye followed by in vitro expansion on the intact globe setup to test the ability to deliver drug and treatment in a live animal.
Materials: Although in vivo treatment does not permit soaking of an entire eye and direct access to the sclera is blocked by conjunctiva and tenon, subconjunctival/subtenon injection is a low-impact surgical procedure that permits drug delivery into the space adjacent to the sclera. Literature on the subconjunctival delivery of mitomycin-C to the sclera indicates that only ~5% of drug present on the surface of sponges is able to diffuse into the sclera, and there is a preferential uptake by the conjunctiva. For this reason, a higher drug concentration than that used in vitro was used to achieve the desired dose in the sclera. Literature reports excellent cell viability with Eosin Y concentrations up to 20 mM and TEOA concentrations up to 450 mM. Our in vivo studies used a solution with 0.289 mM Eosin Y concentration, and 90 mM TEOA concentration, denoted 10x EY from here on. Solutions denoted 10x EY w/PEGDM were a mixture of 10x EY with 10% w/w Poly(ethylene glycol) dimethacrylate. All solutions were adjusted to pH 7.5 and passed through a 0.2 micron filter before use.

Surgical Procedure: The procedures for in vivo drug delivery were conducted at UCSF and were performed on 2–3 week old New Zealand White rabbits. The rabbits were given general anesthesia with 1–5% inhaled isofluorane administered by mask and topical 0.5% proparacaine to the eye. The eye of each animal was sterilized with 5% betadyne. Throughout the procedure the eye was washed with sterile ocular balanced saline solution (BSS).

A minimal procedure using subconjunctival injection (0.6–1.2 mL) placed the drug formulation in contact with the sclera. Eight treated eyes were injected with 10x EY, four treated eyes were injected with 10x EY w/PEGDM, and four control eyes received an
injection of DPBS (Table 5.1). The injection formed a pocket of fluid between the conjunctiva and sclera which remained during the 5 minutes given for diffusion (Figure 5.2a). During this time, the lids were closed over the eye. After the 5 minute diffusion time, the lids were retracted and the eye slightly prolapsed. A circular array of 525 nm LEDs was held around the eye for 5 minutes (Figure 5.2b). The control eyes received irradiation of 2 mW/cm², four 10x EY treated eyes received 2 mW/cm², four 10x EY w/PEGDM treated eyes received 2 mW/cm², and the remaining four 10x EY treated eyes received 6 mW/cm². After irradiation, the animals were sacrificed, and the eyes were enucleated and stored in DPBS on ice until use on the intact globe expansion setup at Caltech.

<table>
<thead>
<tr>
<th>In Vivo Rabbit Drug Delivery</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Set</strong></td>
</tr>
<tr>
<td>A</td>
</tr>
<tr>
<td>B</td>
</tr>
<tr>
<td>C</td>
</tr>
<tr>
<td>D</td>
</tr>
</tbody>
</table>

Table 5.1. Variations for In Vivo Rabbit Treatments and Ex Vivo Expansion
Expansion Testing: Expansion experiments were performed within 48 hours post mortem. The appearance of the eyes (e.g., clarity of the cornea and size of the globe) was unchanged over this time scale. For the expansion experiment, extraocular tissues were carefully removed from the eye and then the eye was placed into DPBS for ~1 hour to equilibrate to room temperature. The eyes were loaded onto the expansion setup where the intraocular pressure was set to 22 mmHg for 1 hour then increased to 85 mmHg for 24 hours (Chapter 2 for details).

5.2.4 Animal Model of Myopia

These experiments in a guinea pig model were conducted in collaboration with Dr. Sally McFadden at the University of Newcastle, NSW Australia. These tests examine the feasibility and safety of surgery, the safety of drug and irradiation, the effect of treatment on development of form deprivation, and the effect of treatment on normal ocular growth.

Materials: All treatment solutions were prepared at pH 7.5 and passed through a 0.2 micron filter to ensure sterility for surgery. The tests used DPBS, 3x EY (0.1 mM EY & 90 mM TEOA in DPBS), and 10x EY.

Pigmented guinea pigs (Cavia porcellus, n = 47) were maternally reared and housed in their natural litters with their mothers in opaque plastic boxes (65 x 45 x 20 cm) with wire mesh lids. Water (supplemented with Vitamin C), guinea pig food pellets, and hay were available ad libitum. Light hoods containing incandescent bulbs evenly diffused through a perpex barrier were suspended 30 cm above each box and switched on a 12 h light/12 h dark cycle. All procedures were approved by The University of Newcastle in accordance
with New South Wales Animal Research Act and were in accordance with National Institutes of Health Guidelines.

Procedures: Animals were anesthetized with Ketamine (50 mg/kg) and Xylazine (5 mg/kg) and if necessary, administered a small dose of Buprenorphine (0.1 mg/kg). The eyes received topical anesthetic as needed. On the right eye, drug was delivered through subconjunctival injection (Figure 5.3), which was previously demonstrated as a successful method in rabbits. Some animals received a sham surgery with injection of DPBS instead of drug (Table 5.2). After subconjunctival injection, 10 minutes was allowed for diffusion of drug formulation into the sclera.

<table>
<thead>
<tr>
<th>Set</th>
<th>Light Protocol</th>
<th>Drug formulation</th>
<th>Form Deprivation</th>
<th># of Guinea Pigs</th>
<th>Day of Enucleation</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>No Irradiation</td>
<td>10x EY</td>
<td>No</td>
<td>3</td>
<td>Immediate</td>
</tr>
<tr>
<td>B</td>
<td>No Irradiation</td>
<td>No Treatment</td>
<td>Yes</td>
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<td>17 days post surgery</td>
</tr>
<tr>
<td>C</td>
<td>3 Trisections</td>
<td>3x EY</td>
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<td>14</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>3 Trisections</td>
<td>10x EY</td>
<td>No</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>Circumferential</td>
<td>10x EY</td>
<td>No</td>
<td>8</td>
<td>30 days post surgery</td>
</tr>
<tr>
<td>F</td>
<td>Circumferential</td>
<td>DPBS (sham)</td>
<td>No</td>
<td>8</td>
<td></td>
</tr>
</tbody>
</table>

Table 5.2. Treatment Variations for *In Vivo* Guinea Pig Studies
After the 10 minute diffusion time, the right eyes of Sets C-E were prolapsed and irradiated in two different manners. One group of animals had a superficial suture placed at the limbus for traction while prolapsing the eye (Sets C, D). The eye was irradiated with an LED light source for 5 minutes at each of 3 trisections (Figure 5.4a). The second group of animals had a piece of elastic placed around the eye to hold it prolapsed (Set E, Figure 5.4b). While prolapsed in this manner, a circular array of LEDs was placed around the eye for 5 minutes (Figure 5.4c, d). We built the light sources from 525 ± 16 nm LEDs to provide 6–8 mW/cm² at the plane of the sclera; the light for trisection illumination consisted of three 5 mm LEDs aligned to irradiate a 120 degree section of the eye while held a distance of ~8 mm from the eye, and the light for circumferential illumination consisted of 2 rows of twelve 3 mm LEDs ~2 mm from the scleral surface that could irradiate 360 degrees of the eye.

Figure 5.3. Controlled subconjunctival injection of 10x EY delivers treatment to the eye. 10x EY is seen as pink on the right side, and locations where 10x EY has not yet been delivered are seen as grey on the left side.
After irradiation, the eyes were placed back in the normal position and washed with antibiotic eyedrops. The animals were placed back with their mothers after surgery and monitored to observe behavioral responses.

Animals from Set A were immediately euthanized and the eyes were enucleated. The eyes were examined for the presence of Eosin Y in the sclera.
The animals from Sets B and C included form-deprivation studies. Diffusers were secured with velcro over the right eye when the animals were ~6 days old and the fellow eye was left untreated (Figure 5.5). This was 2–3 days after surgery of animals in Set C. The animals were exposed to a 12h/12h light/dark cycle, and the diffusers were removed for 50–90% of the dark periods overnight. Diffusers were also removed during measurements.

![Figure 5.5. Method of attaching a diffuser in the guinea pig. (A) Position of the Velcro arcs. Dotted lines indicate the horizontal and vertical meridians used in retinoscopy. (B) The diffuser held in place by a matching Velcro ring attached to its base (Howlett 2006)](image)

Animals from Sets D, E, & F did not receive diffusers and they were monitored to observe normal growth of the eye.

Measurements were made before surgery, and then periodically after surgery to track changes in eye shape throughout form deprivation and normal growth. Corneal power was measured using IR videokeratometry. The animals were cyclopleged (i.e., dilated) with 2 drops of 1% cyclopentolate, and refractive error was measured using streak retinoscopy. Finally, the animals were anesthetized with 2% isoflurane in oxygen and the axial ocular parameters were measured using high-frequency ultrasound (20 MHz).
Within 2 days of the last ocular measurements, guinea pigs were euthanized and the eyes were prepared for histology. A strip of tissue was dissected from the eye cup, fixed overnight in 4% glutaraldehyde, imbedded in resin, cut in 1 μm sections, and then mounted and stained.

5.3 Results

5.3.1 In Vitro Application and In Vitro Expansion

Treatment with GA was performed as a positive control to demonstrate the ability of crosslinking to prevent creep and the results have previously been discussed in Chapter 2. Motivated by the advantages of using a visible light activated crosslinking system, we chose EY/TEOA for these studies. Digitized images from the expansion studies were analyzed to measure the ocular dimensions labeled in Figure 5.6. Over the 24 hour period, control eyes expand continuously along every dimension (Figure 5.7). This is expected due to the high pressure which induces creep. The treated eyes resist expansion along SP, ED, and SL—dimensions associated with the sclera. Expansion along dimensions associated with the cornea (CP, CD, and CL) increase in the same manner for treated and control eyes. Because the corneal epithelium remained intact during treatment, it provided a protective layer that prevented treatment of the cornea. Removal of this layer in order to treat the cornea will be discussed in Chapter 6. Because we are currently interested in the treatment’s ability to strengthen sclera for degenerative myopia, we will focus on results of SP, ED, and SL expansion (all components of the sclera).
Treatments tested with a high-intensity, broadband arc lamp source, and with a low-intensity LED light source both show similar results after 24 hours (Figure 5.8). Further reduction in the intensity may be possible using a light source more in tune with the absorption peak of EY (514 nm). The use of low light doses (5 minutes, 6 mW/cm²) of visible wavelength may avoid the cytotoxic effects on the retina that were seen with larger doses of UV (30 minutes, 3 mW/cm²) used by Wollensak.
Figure 5.7. Rabbit eyes subjected to a low prestress similar to normal intraocular pressure (22 mmHg) followed by a high (85 mmHg) pressure for 24 hours show the ability of eyes treated with 1x EY to resist creep. The sclera of treated eyes remains stable (top row) while the cornea expands indistinguishably from control cornea (middle row). For values of the overall dimensions, refer to Table 2.2.
5.3.2 Biocompatibility

Biocompatibility studies were performed on albino rabbit eyes because the lack of pigmentation in these eyes allows for easy visualization of toxic or inflammatory responses. In all of the eyes we operated on, there was some observed swelling and inflammation for 2 days following the procedure. This was consistent with what would be expected to result from the surgical procedure itself. There were no clinical signs of pain or inflammation in any of the eyes 3 days after the procedure and on each examination thereafter.
Histological examination revealed that there was mild inflammation and scarring along the conjunctival-sclera junction in the surgical area of all the eyes. The irises, retinas, and ciliary bodies were all normal in all experimental groups. There was no significant difference in the sclera of treated (Figure 5.9a) and control eyes (Figure 5.9c), indicating that the mild inflammation and scarring which occurs is a result of the surgery and not of the treatment. Likewise, the viability of cells in the nearby tissues of the treated eyes matches that of normal eyes.
Figure 5.9. Histopathological examination of 1x EY Treatment (Group 1—a, b) and DPBS control vehicle (Group 2—c, d) treated rabbit eyes. The arrows mark the boundary between the sclera and conjunctiva (sclera is to the left of the arrow). All micrographs used 20x magnification. The treated, irradiated area of Group 1 and Group 2 rabbits shows mild inflammation and moderate scarring (darker blue line of staining by the arrow) where the conjunctiva was retracted. For comparison, the untreated, non-irradiated area of a Group 1 and Group 2 rabbits where conjunctiva was not retracted shows normal sclera and conjunctiva with no inflammation or scarring. (Greater thickness of the sclera relative to (a) and (c) is characteristic of the location of the section.)
5.3.3 In Vivo Application and In Vitro Expansion

Average values of changes along ocular dimensions indicate that all injections except the control decrease the expansion of the sclera (Figure 5.10). Values for expansion along SP and ED are significantly smaller compared to controls for the low-intensity treatment, while all values for the high-intensity treatment are significantly smaller than controls. Significance with $p < 0.05$ was determined by comparing values from treatment and control groups using an unpaired t-test. After 24 hours of elevated pressure, in vivo treated eyes have an ocular stability comparable to that of the in vitro treated eyes (Figure 5.8). This proves that the subconjunctival injection delivers drug to the sclera, and the 5 minute diffusion time is sufficient for 10x EY to penetrate into the live sclera. In addition, the circumferential irradiation with LEDs is able to activate the treatment around the eye.
5.3.4 Animal Model of Myopia

Using a guinea pig model, data was obtained regarding drug delivery, toxicity, and tolerance of surgical procedures. After surgery, there was minimal inflammation of the conjunctiva that disappeared within 2–3 days. The eyes had a normal pupil response and clear ocular media which allowed for streak retinoscopy measurements. Gross ocular function (pupillary reflexes, response to light, blink reflex) appeared normal. Behaviorally, the animals moved about the habitat normally and had normal eating and drinking habits.

Observations of the sham surgery controls indicated that the surgical procedure was well-tolerated by the eyes. The eyes receiving the 3x EY and 10x EY formulations demonstrated no evidence of toxicity problems. Tissue sections from treated and fellow
eyes showed that the sclera was structurally normal (Figure 5.11). The sclera, choroid and retinal pigment epithelium (RPE) had no signs of toxicity from the treatment. There were normal RPE cells and depigmented RPE cells in the treated and untreated eye sections. The sclera of treated and untreated eyes is indistinguishable. Although the retina was removed before fixing the tissue, the overall retinal thickness was within the normal limits and no signs of retinal toxicity were observed. These important findings support our hypothesis that the treatment is safe based on EY/TEOA literature,\textsuperscript{1-11} light- and drug-penetration calculations, and rabbit histology.

Of the three eyes enucleated immediately after treatment (Set A), all showed pink staining from Eosin Y over the entire sclera, including at the posterior pole, indicating that the formulation can be delivered to the entire sclera following subconjunctival injection.
Results of normal form-deprivation with untreated eyes (Set B) are presented along with results for form-deprivation of 3x EY treated eyes (Set C). Measurements of refractive state before surgery indicate that the guinea pigs are hyperopic, which is expected for their age (Figure 5.12). Immediately before beginning form-deprivation (2 days after surgery), the treated (3x + FD) and fellow control eyes (3x Fellow) are the same, indicating that surgery had no effect on refractive error. For normal form-deprivation, the differences of refractive error between the form deprived (FD) and fellow eye (Fellow) are -4.04 ± 0.667 D on the first measure (day 7), and -5.12 ± 0.659 D on the second measure (day 11). Nearly identical changes are seen in the treated animals with differences of -4.11 ± 0.675 D and -5.23 ± 0.612 D on the matching days. Measurement of ocular length (from the front
of the cornea to the back of the sclera) by ultrasound also reveals similar behavior in the treated and untreated animals (Figure 5.12b). On day 7, the myopic eye was 111 ± 20.4 μm greater in length than the fellow eye. By day 11, this length difference reduced some in the untreated animals, but remained the same in the 3x EY treated animals. The similarities between the 3x EY treated (Set C) and the normal animals (Set B) indicates that this treatment does not have an effect on the eye. We hypothesize that insufficient EY diffused into the tissue, motivating experiments with 10x EY (below).

Although this treatment was not able to prevent myopia, the results were encouraging due to the lack of cytotoxic effects using 3x EY and irradiation, and the resilience of animals to the surgery. Before examining higher doses in form-deprived animals, we began tests of higher doses in normal eyes to observe if they could tolerate the dose (Sets D & E). At this time, analysis from Sets E and F is incomplete and only results from the other sets are presented.

Eyes from Set D received the same irradiation protocol as those from Set C, but were given higher doses of drug (10x EY instead of 3x EY). Measures of refractive error indicate that 2 days after surgery there is a difference between the treated eye and untreated fellow eye of -3.11 ± 0.714 D (Figure 5.13a). The treatment causes the eye to become more myopic. Over the course of the experiment, the treated eye becomes more hyperopic. The fellow eye emmetropizes normally during this period. The 10x EY treatment also causes an increased ocular length, and the difference between treated and untreated fellow eyes reduces over time (Figure 5.13b). These initial differences were not seen in the 3x EY
treated eyes and they indicate that significant changes have occurred due to treatment with 10x EY.

The change in ocular length is examined in greater detail using ultrasound biometry to evaluate all the ocular dimensions that contribute to ocular length (Figure 5.14). The cornea and anterior chamber thickness (CAC) grows normally for both eyes (Figure 5.14a). The lens grows normally despite an initial difference at day 2 (Figure 5.14b). The variability in day-to-day lens thickness suggests that the uncertainty in the measurement is greater than the error bars indicate. The vitreous chamber elongates more slowly in the treated eye than in the untreated eye (Figure 5.14c). There is no difference in retinal thickness (Figure 5.14d). The choroid and sclera are both thicker in the treated eye (Figure 5.14e).
The sum of these individual components gives the ocular length reported in Figure 5.13b:

\[ \text{CAC + Lens + Vit + Ret + Scl + Chr} = \text{OL}. \]

\[ 1.06 \text{ mm} + 3.53 \text{ mm} + 3.02 \text{ mm} + 0.16 \text{ mm} + 0.11 \text{ mm} + 0.11 \text{ mm} = 7.99 \text{ mm}. \]

The slight differences in corneal power dissipate over the growth period (Figure 5.14e).

The differences in the sclera, choroid, and vitreous chamber of the treated and fellow eyes persist over 15 days of observation. Choroid thickness is known to increase with inhibitory growth signals, and the drug treatment may have triggered an inhibitory response. The initial change in vitreous chamber depth may be explained by crosslinking of the sclera in an extended state. The intraocular pressure increases during prolapsing, which could induce stretching of the sclera. After prolapsing, the pressure decreases, and the sclera relaxes back to normal. However, in a treated eye, the stretched state of the sclera might be crosslinked in place, causing noticeable shape differences. Further tests such as MRI may be capable of examining the shape of the eye before and after prolapsing, with and without treatment.

The data also suggests that the cornea grows in a normal manner in a treated eye despite the abnormal changes in vitreous chamber depth. This is also seen with the normal growth of the lens. The growth of the cornea and lens may not be coupled to axial length. Using this method of crosslinking tissue, whether it is cornea or sclera, might enable researchers to
determine if there is a coupled feedback for growth of the ocular components in these animals.

**Figure 5.14.** The ultrasound measurements are analyzed to measure corneal thickness and anterior chamber depth (CAC), lens thickness (Lens), vitreous chamber depth (Vit), retina thickness (Ret), choroid thickness (Chr), and sclera thickness (Scl) as F) illustrates. There are no differences between the 10x EY treated and untreated fellow eyes for A) CAC, B) Lens, D) posterior layers, and E) corneal power. C) The vitreous chamber elongates faster in the untreated fellow eye than in the treated eye and D) the sclera and choroid are thicker in treated eyes.
5.4 Summary

Were it possible to retard or prevent abnormal axial elongation of the globe in degenerative myopia, visual loss might be prevented. Use of the expansion model in this study has allowed us to measure the progressive enlargement of the eye due to creep in the sclera. The ability of 1x EY and 10x EY to halt expansion in vitro in rabbit eyes indicates that the change in tissue properties upon treatment might prevent creep in vivo. Results from the biocompatibility studies in rabbits and guinea pigs show only minor inflammation from the surgery, and no adverse responses due to treatment concentrations up to 10x EY. Results from in vivo guinea pig studies demonstrate that the treatment with 3x EY did not alter ocular shape or prevent form-deprivation myopia. However, the higher dose of 10x EY did substantially alter ocular parameters during normal growth, possibly due to elevated intraocular pressure during prolapsing at the time of irradiation. The experiments establish protocols that may be extended to form-deprivation studies of 10x EY, perhaps with modification of the irradiation step to ensure that the globe is at normal intraocular pressure. Future treatments of the entire eye, or specifically the posterior pole, are also recommended to test their ability to prevent form-deprivation myopia in the guinea pigs.
BIBLIOGRAPHY


