

# Delivery from Episcleral Explants

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**PURPOSE.** To assess the impact of an episcleral explant on transscleral delivery.

**METHODS.** New Zealand White rabbits were given a periocular injection of sodium fluorescein (fluorescein, 376 Da) or an episcleral explant loaded with fluorescein. Two types of explants were tested: (1) a rigid polyethylene device, impermeable on one side and open to the sclera on the other, that contained compressed pellets of fluorescein and was sutured loosely (apposition group) or tightly to indent the sclera (indentation group) and (2) flexible refillable silicone explants also open to the sclera that were secured by suturing, to form a sealed episcleral chamber that was filled with a fluorescein solution. Ocular and plasma fluorophotometry were performed at several time points, and histology was performed to evaluate the effect of explants on the periocular tissue.

**RESULTS.** Within 20 minutes of a periocular injection of fluorescein, peak fluorescence was visible in the anterior chamber (AC) and at later time points was displaced toward the retina; at all time points, the highest fluorescence was in the AC. For the polyethylene device indentation group, peak fluorescence was in the retina and posterior vitreous and spread to the AC over time. For the apposition explant group, two peaks of fluorescence were seen initially, one in the retina and posterior vitreous and one in the AC. The area under the concentration time curve (AUC  $\pm$  SE) for fluorescein concentration was  $144.4 \pm 15.1 \mu\text{g} \cdot \text{h/mL}$  for the retinal peak and  $43.6 \pm 7.1 \mu\text{g} \cdot \text{h/mL}$  for the posterior vitreous peak after injection of 5 mg of fluorescein into a silicone explant, compared with a retinal peak of  $3.9 \pm 0.3$  and a posterior vitreous peak of  $0.99 \pm 0.26 \mu\text{g} \cdot \text{h/mL}$  after periocular injection of 5 mg of fluorescein ( $P < 0.01$  for each). Peak plasma fluorescein levels were significantly reduced in the explant group compared with periocular injection.

**CONCLUSIONS.** An episcleral explant facilitates diffusion of fluorescein through the sclera resulting in high levels in the retina and posterior vitreous; levels are markedly increased compared

with periocular injection of the same amount of fluorescein. It also reduces peak plasma levels indicating reduction of systemic absorption. This procedure provides a new approach that can be combined with sustained-release preparations to optimize delivery of agents to the retina and choroid while minimizing the potential for systemic toxicity. (*Invest Ophthalmol Vis Sci.* 2006;47:4532–4539) DOI:10.1167/iov.06-0030

As progress is made in understanding the pathogenesis of diseases, targets for therapeutic intervention emerge. For instance, several lines of evidence have implicated vascular endothelial growth factor (VEGF) as a critical stimulus for both retinal and choroidal neovascularization,<sup>1–4</sup> resulting in development of VEGF antagonists for treatment. Proof of concept has been provided by clinical trials that showed that repeated intravitreal injections of pegaptanib, an aptamer that binds VEGF<sub>165</sub>, reduces severe loss of vision in patients with neovascular age-related macular degeneration (NVAMD).<sup>5</sup> The effect of intravitreal injection of other VEGF antagonists is being tested.

Intravitreal injection allows rapid delivery of agents to the retina and choroid, but carries risk of endophthalmitis and retinal detachment. Also, it results in high peak levels of drug separated by troughs that may be below the therapeutic threshold, which is not an ideal delivery profile for most agents. Less-invasive routes of delivery that achieve sustained therapeutic drug levels could provide major benefits.

Periocular injections carry substantially less risk than intraocular injections, but for many agents they do not provide sufficient intraocular drug levels to achieve the desired effects. This problem is particularly evident in antibiotic treatment for endophthalmitis, for which intraocular injections are essential.<sup>6</sup> Both periocular and intravitreal injections of steroids can provide benefit for some conditions, such as some types of ocular inflammatory diseases,<sup>7,8</sup> and it is not known for certain which route is superior. However, for other conditions, such as diabetic macular edema, intravitreal injections of triamcinolone acetonide are superior to sub-Tenon injections,<sup>9,10</sup> and higher vitreous concentrations of triamcinolone acetonide are achieved after intravitreal compared with sub-Tenon injections.<sup>11</sup> A potential barrier to adequate drug delivery after periocular injection is penetration through the sclera, determined by size and other characteristics of the molecule<sup>12</sup>; but even molecules that penetrate well may have little opportunity to do so because of diffusion away from the scleral surface and absorption into the systemic circulation. This problem could be limited by placement of the drug in an impermeable reservoir that provides free access to the sclera and prevents drug from diffusing away thereby maintaining a concentration gradient favoring penetration through the sclera. In this study, we tested the concept of unidirectional transscleral drug delivery by measuring intraocular fluorescence in vivo after implanting sodium fluorescein (fluorescein)-loaded episcleral devices (Sclcl; 3T Ophthalmics, Irvine, CA) and comparing the intraocular fluorescence achieved after periocular injection of the same amount of sodium fluorescein.

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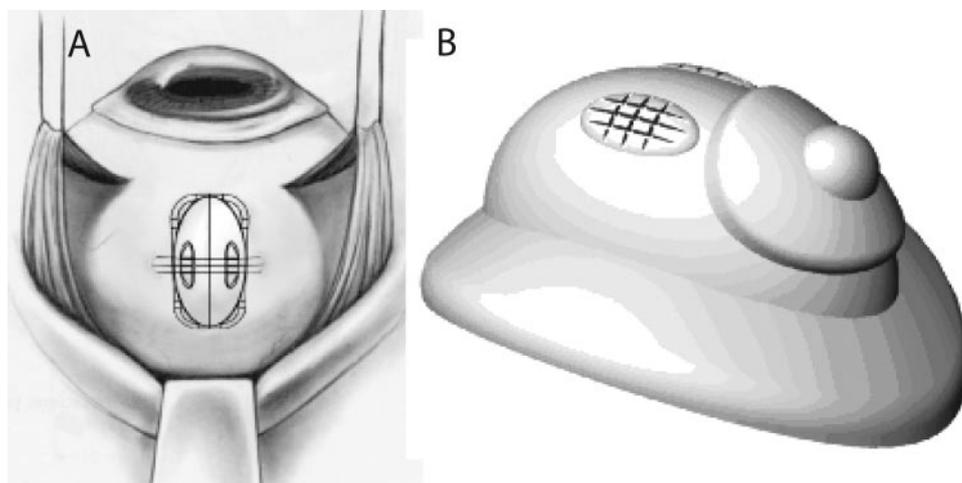
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**FIGURE 1.** Schematic of episcleral explants. (A) The appearance of the rigid polyethylene episcleral explant sutured to the globe. (B) The refillable silicone episcleral explant is flexible and forms a seal with the globe without tight suturing to the globe.

## MATERIALS AND METHODS

### Treatment Groups and Assessments

All the experiments were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Two sets of experiments were conducted: one with rigid polyethylene devices and the second with flexible silicone devices.

In the first set of experiments, New Zealand White rabbits (8–10 lb.) were anesthetized and had a rigid polyethylene device (Fig. 1A) sutured to the sclera of one eye to provide apposition (apposition group,  $n = 4$ ) or sutured tightly causing the rim of the device to indent the sclera (indentation group,  $n = 4$ ). Once the devices were in place, the sclera formed one wall of the compartment and was the only surface through which diffusion could take place. Nine milligrams of fluorescein sodium (Sigma-Aldrich, St. Louis, MO) was compressed into pellets by a manual press system (International Crystal Laboratories, Garfield, NJ) with 10 ft<sup>2</sup> of torque applied to a die set of 4 mm. Fluorescein pellets were placed in each of the explants. A third group of rabbits ( $n = 3$ ) received a periocular injection of 9 mg of fluorescein in 0.09 mL of phosphate-buffered saline (PBS). At baseline and at several time points after implantation or injection (0.25, 1, 3, 12, 24, and 48 hours, 5 and 7 days), ocular and plasma fluorescein levels were measured with an ocular fluorophotometer (FM-2 Fluorotron Master; Occumetrics, Mountain View, CA). Measurements were performed at least twice, and two consistent measurements were averaged to provide one data point for analysis.

In the second set of experiments, New Zealand White rabbits (8–10 lb) were anesthetized, and a flexible refillable silicone device (Fig. 1B) was sutured to the sclera. Fluorescein was diluted at 10% in a water-soluble viscous vehicle (Bion Tears; Alcon, Fort Worth, TX) composed of dextran (0.1%) and hypromellose (0.3%). A volume of 0.05 mL (5 mg) of this solution was injected into the periocular space in close apposition to the sclera ( $n = 3$ ) or into the empty reservoir of the preplaced silicone device ( $n = 4$ ) while venting with another needle. Ocular and plasma fluorophotometry were performed at baseline and at 1, 3, 6, 24, and 48 hours and 5, 7, 14, 28, and 52 days after the procedure.

External ocular examinations and indirect ophthalmoscopy were performed during the first 2 weeks and before euthanasia, 2 months after implantation. Eyes, including the attached explant and overlying conjunctiva, were rapidly removed and frozen in optical cutting temperature embedding solution (Miles Diagnostics, Elkhart, IN). Ocular sections (10  $\mu$ m) were stained with hematoxylin and eosin and examined by light microscopy.

### Surgical Procedures

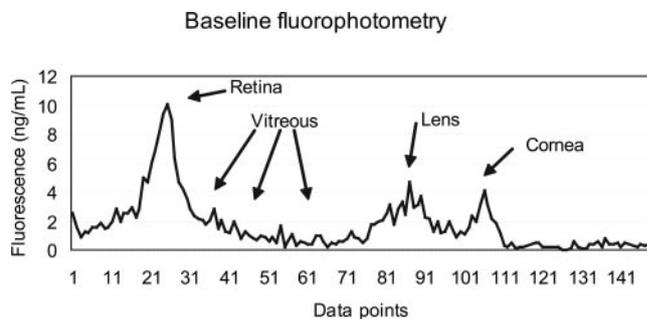
Periocular injections were performed with a tuberculin syringe connected to a 25-gauge needle by proceeding along the globe and then

injecting adjacent to the sclera, posterior to the equator of the eye. The injection site was compressed as the needle was removed to try to minimize the reflux of fluorescein.

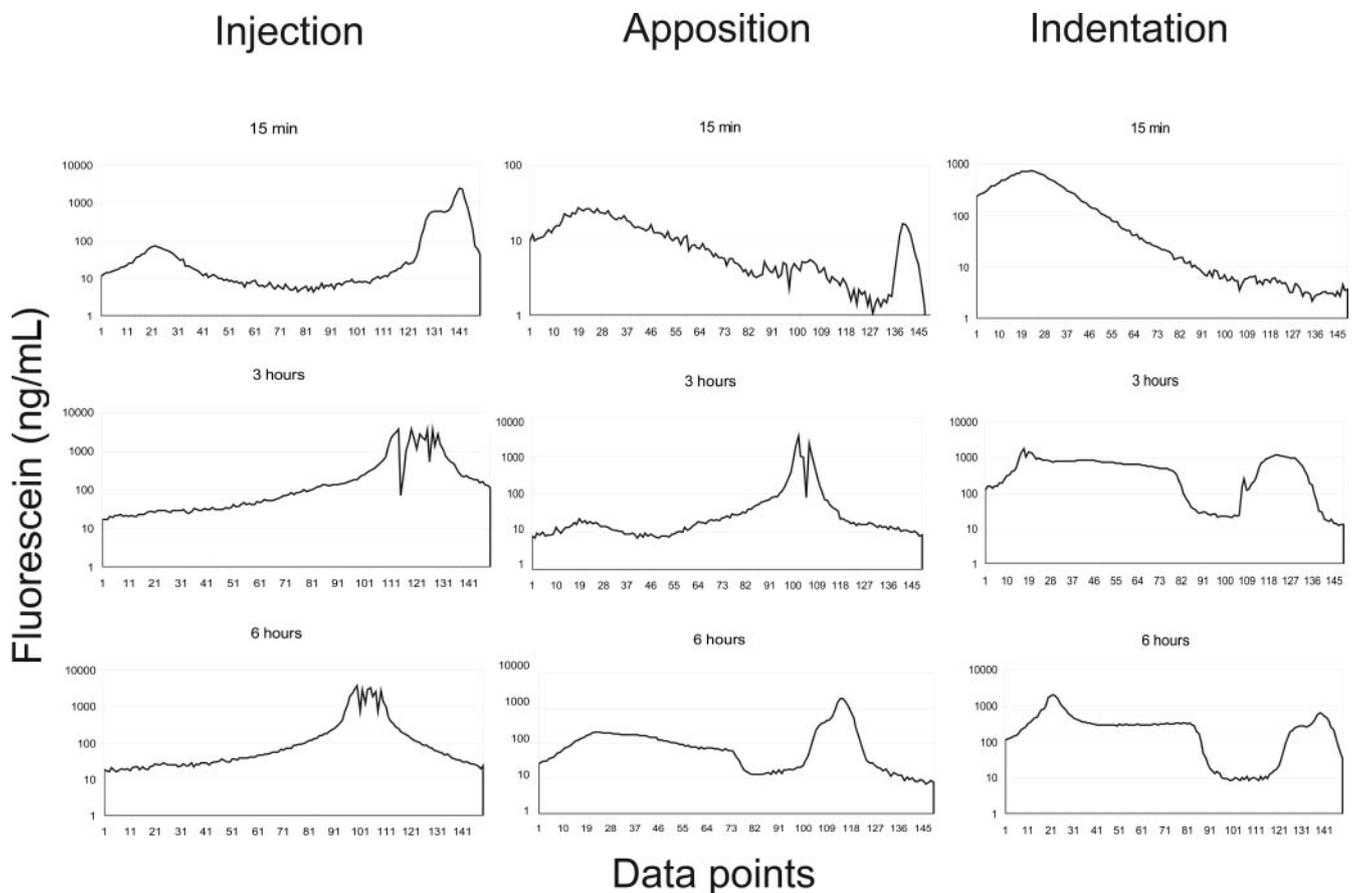
For implantation of polyethylene devices, a conjunctival incision was made and the surface of the sclera was exposed and cleaned. Two episcleral polyester sutures (6-0 Ti-Cron; Synture, Norwalk, CT) were placed on each side of the device, and a drop of cyanoacrylate adhesive was applied to each corner of the device to hold it in place while the sutures were secured. Sutures were tied tightly to cause indentation of the sclera with the rim of the device (indentation group) or less tightly to cause apposition, but no indentation (apposition group). The conjunctiva was closed over the explant with glycolide-lactide suture (Polysorb; Synture). The same procedure was used for implantation of silicone devices, but because of the compressibility of the devices, a scleral seal was obtained without indentation, after which fluorescein solution was injected into the reservoir without leakage.

### Data Analysis

A baseline ocular scan was performed for each eye, for which there are peaks from autofluorescence in the retina and adjacent retinal pigmented epithelium, lens, and cornea (Fig. 2). For each posttreatment scan, the baseline scan was subtracted from the posttreatment fluorescence and, by using a standard curve, the fluorescein concentration in the retina and the posterior vitreous were obtained. The posterior vitreous level was calculated by taking the mean of eight measurements 0.25 mm apart between 3 and 5 mm anterior to the retina. The retinal and posterior vitreous fluorescein levels were obtained at each time point and were plotted to generate the area under the fluorescein concentration-time curve (AUC) in nanograms per hour per milliliter for various time intervals.



**FIGURE 2.** Baseline fluorophotometry scan. A baseline fluorophotometry scan shows peaks from autofluorescence in ocular structures. Anatomic components of the eye are identified by their position along the axial diameter of the eye.



**FIGURE 3.** Fluorophotometry scans after periocular injection of fluorescein or placement of a rigid polyethylene explant containing a fluorescein pellet. Fluorophotometry scans were performed at several time points after New Zealand rabbits received a periocular injection of 9 mg fluorescein in 0.09 mL of PBS (injection group,  $n = 3$ ) or had a rigid polyethylene device sutured to the sclera of one eye to provide apposition (apposition group,  $n = 4$ ) or sutured tightly to indent the sclera (indentation group,  $n = 4$ ). For each of the three groups, representative scans taken at 15 minutes and 3 and 6 hours are shown. The 15-minute scan after periocular injection shows a retinal peak and a larger peak in the anterior chamber, and at subsequent time points there is only an anterior chamber peak, which suggests that some fluorescein enters by transscleral diffusion, but a substantial amount enters by diffusion on the outside of the globe and entry through the cornea. The same is true in the apposition group, although at the 6-hour time point, it appears that there is substantial fluorescein in the posterior vitreous. In contrast, the indentation group shows a different pattern of scan morphology. At the 15-minute time point, there is a very large retinal peak and no anterior chamber peak, suggesting that most fluorescein enters through the sclera. At later time points, the peak is still highest posteriorly, suggesting a posterior-to-anterior diffusion gradient in the eye.

### Pharmacokinetic and Statistical Analyses

Noncompartmental analysis was performed with commercial software (WinNonlin Pro 4.1; Pharsight Corp., Mountain View, CA). Pharmacokinetic data were analyzed with statistical software (Stata 7.0; Stata, College Station, TX). The AUC was calculated with the linear trapezoidal rule from 0 to 48 hours ( $AUC_{0-48h}$ ), 0 to the last time point ( $AUC_{0-last}$ ), and 0 to infinity ( $AUC_{0-infinity}$ ; based on observed data). ANOVA with Bonferroni correction was used for multiple comparisons. A *t*-test was used for two-sample comparisons. Type I error was set below 0.05 for statistical significance.

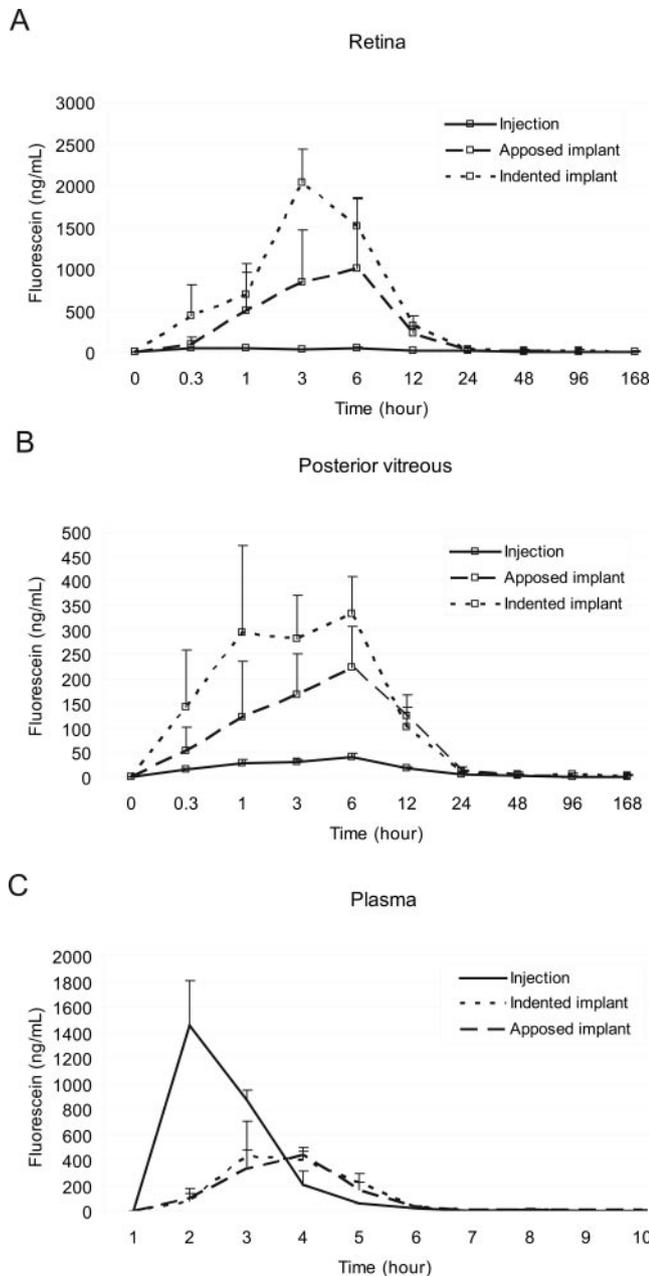
### RESULTS

#### Ocular Fluorophotometry as a Tool for Quantitative Assessment of Transscleral Penetration of Fluorescent Molecules

Ocular fluorophotometry after intravitreal injection of fluorescein is a valuable tool for assessment of the blood-retinal barrier.<sup>13</sup> Instead of injecting fluorescein intravenously and measuring penetration into the eye from the systemic circulation, we used ocular fluorophotometry to measure transscleral

penetration of fluorescein. Figure 3 shows the different patterns of fluorescein distribution that were consistently seen in the first set of experiments. Fifteen minutes after periocular injection of 9 mg of fluorescein, there was approximately a 10-fold increase in fluorescein in the retina compared to baseline (Fig. 3, column 1, top). There were also elevated levels in the posterior vitreous, but substantially lower than those in the retina. The highest levels in the eye, roughly 10-fold higher than those in the retina, were in the anterior chamber. At 3 and 6 hours after periocular injection (Fig. 3, middle and bottom), there were persistently high levels of fluorescein in the anterior chamber, disappearance of the retinal peak, and a clear anterior to posterior concentration gradient. These observations indicate, as has been suggested,<sup>14</sup> that one route of entry of small molecules after periocular injection is by diffusion anteriorly on the outside of the eye, entry into the anterior chamber through the cornea, and diffusion posteriorly within the eye.

Fifteen minutes after a polyethylene device containing a 9 mg fluorescein pellet was tightly sutured to the sclera (Fig. 3; indented group, top), there was a large retinal peak, 10-fold higher than that seen after periocular injection of the same

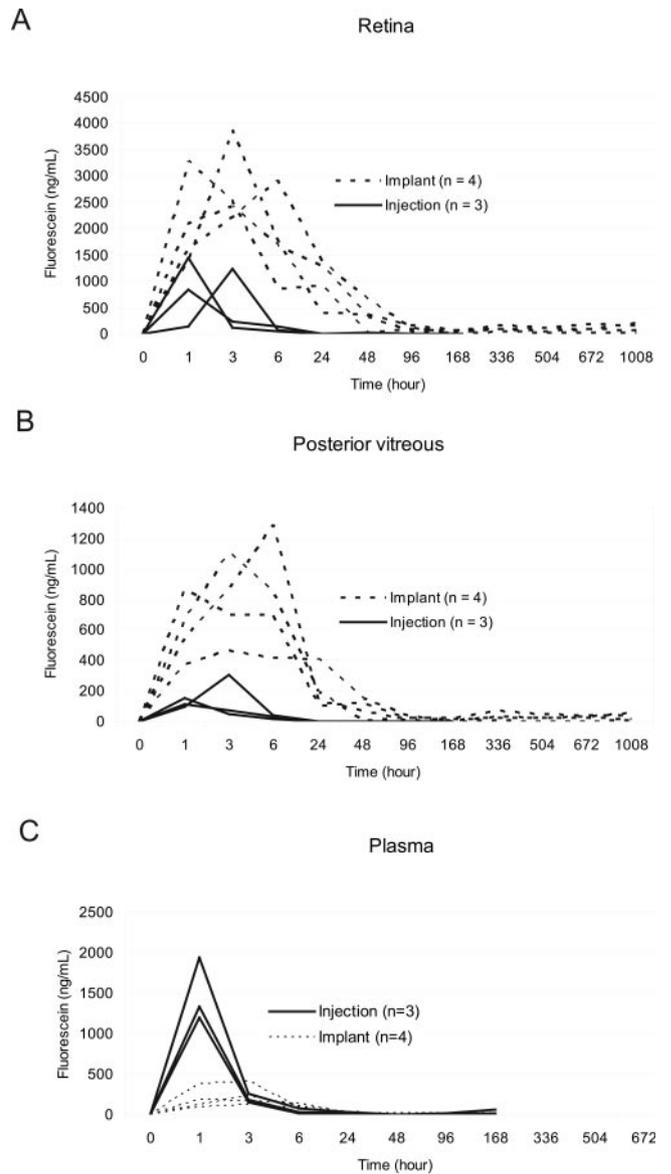


**FIGURE 4.** Levels of fluorescein in the retina, posterior vitreous, and plasma over time after periocular injections or placement of rigid exoplants. Fluorophotometry scans were performed at several time points on rabbits in each of the three groups, as described in the legend to Figure 3. Each point shows the mean ( $\pm$ SD) fluorescein concentration in the retina (A), posterior vitreous (B), or plasma (C). Mean fluorescein levels in the retina and posterior vitreous were much higher in the two explant groups for at least 12 hours, whereas plasma levels peaked earlier and at a much higher level after periocular injection of fluorescein than after either of the exoplants.

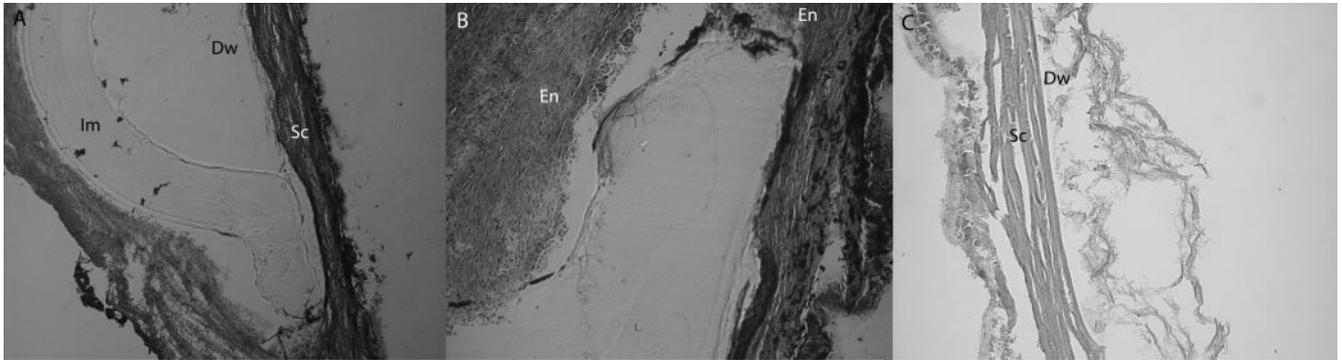
amount of fluorescein. Levels in the anterior chamber were low. At 3 and 6 hours after implantation (column 3, middle and bottom), there were high levels in the retina/choroid, and high levels in the vitreous and anterior chamber with a posterior-to-anterior concentration gradient.

Loosely suturing a device containing a 9-mg fluorescein pellet to the sclera (Fig. 3, apposition group) resulted in a fluorophotometry pattern that had some similarities to the

indented explant group; but, at later time points, levels were higher in the anterior chamber than the posterior part of the eye, similar to the pattern seen after periocular injection of fluorescein. In the second group of experiments in which fluorescein was injected into preplaced flexible silicone exoplants, the fluorophotometry patterns were similar to those seen with indentation of the rigid exoplants, suggesting that a tight seal to the sclera was achieved with the flexible silicone exoplants.



**FIGURE 5.** Levels of fluorescein in the retina, posterior vitreous, and plasma over time after injection of fluorescein into the periocular space or into a preplaced flexible explant. Rabbits had injection of a viscous solution containing 5 mg of fluorescein into the periocular space (injection group,  $n = 3$ ) or into a preplaced flexible silicone device (explant group,  $n = 4$ ). Fluorophotometry scans were performed at several time points, and the fluorescein concentrations over time in the retina (A), posterior vitreous (B), and plasma (C) were plotted for each rabbit. Levels in the retina and posterior vitreous were substantially above baseline for 96 hours or longer for the flexible exoplants compared with less than 6 hours for periocular injections. Plasma levels were higher and peaked earlier after periocular injections than after injections into the flexible exoplants.



**FIGURE 6.** Histology of a rigid exoplant from the tightly sutured, indented group 2 months after implantation. (A) Light microscopy of ocular sections through a rigid implant (Im) stained with hematoxylin and eosin shows lack of inflammation and fibrous tissue within the reservoir of the exoplant resulting in an unimpeded diffusion window (Dw) through the sclera (Sc). (B) Note the tight apposition between the device base and the sclera (Sc) so that the encapsulating fibrous tissue (En) surrounds the outside of the exoplant, but does not enter between the device and the sclera. (C) A region of sclera (Sc) where a refillable silicone implant had been located shows loose extracellular matrix in the region of the previous diffusion window (Dw).

### Levels of Fluorescein in the Posterior Vitreous and Plasma Over Time after Periocular Injections or Episcleral Explants

Measurements of fluorescein levels in the retina, posterior vitreous, and plasma were performed and plotted over time after injection or implantation for each of the experimental groups. In the first group of experiments, fluorescein levels in the retina and posterior vitreous were higher with an indented or apposed device than with a periocular injection at all time points up to 12 hours and by 24 hours returned to baseline (Figs. 4A, 4B). Plasma fluorescein levels peaked earlier and at a much higher level after periocular injection of fluorescein than after either of the devices (Fig. 4C).

Compared with periocular injection of a viscous solution containing 5 mg of fluorescein, injection of the same solution into a preplaced flexible silicone device resulted in significantly higher levels of fluorescein in the retina and posterior vitreous at all time points (Fig. 5A, 5B). Levels were substantially above baseline for 96 hours or longer for the flexible devices compared with less than 6 hours for periocular injections. Injection of 5 mg of fluorescein in viscous vehicle into flexible explants resulted in higher levels than those seen with implantation of rigid devices containing 9-mg pellets of fluorescein, possibly because the viscous vehicle sustained levels within the devices for longer periods or because there was less loss of fluorescein from the preplaced flexible devices. The latter is suggested by the fairly reproducible curves obtained with four different flexible explants (Figs. 5A, 5B). Plasma levels were higher and peaked earlier after periocular injections than after injections into the flexible explants (Fig. 5C).

Pharmacokinetic analysis of the first group of experiments (Table 1) showed that compared to the periocular injection group, the peak concentration of fluorescein ( $C_{max}$ ) obtained in the retina was significantly greater in the indented device group, but not in the apposed device group. The peak concentration in the posterior vitreous was not significantly higher. The  $AUC_{0-48h}$  provides an assessment of the total amount of fluorescein that entered the retina over the first 48 hours, whereas the  $AUC_{0-last}$  and  $AUC_{0-infinity}$  provide assessments of the total amount of fluorescein that entered the retina for the duration of the study. Compared with the periocular injection group, each of these values was significantly greater in the indented device group, but not the apposed device group. The peak plasma fluorescein concentration was significantly less in both of the device groups than in the periocular injection

group, but there was no significant difference in plasma  $AUC_{0-last}$  among the three groups. This result suggests that the explants delay entry of fluorescein into the systemic circulation and thus decrease the peak plasma level, but they do not reduce the total amount that enters the circulation.

Pharmacokinetic analysis for the second group of experiments showed that the peak fluorescein concentration in the retina or in the posterior vitreous was significantly higher after injection of 5 mg of fluorescein into the preplaced flexible device compared to injection of 5 mg into the periocular space (Table 2). Both compartments also showed a significant elevation in the  $AUC_{0-48h}$ ,  $AUC_{0-last}$ , and  $AUC_{0-infinity}$  in the device group compared to the injection group. The differences between the groups in the  $AUC_{0-last}$  and  $AUC_{0-infinity}$  suggests that roughly 37-fold more fluorescein entered the retina when injected into the device, and roughly 44-fold more fluorescein made it into the posterior vitreous. The  $AUC_{0-48h}$  was approximately one third of the  $AUC_{0-last}$  or  $AUC_{0-infinity}$  for the device group, but they were all essentially the same for the periocular injection group, indicating that all the fluorescein that is going enter the eye after periocular injection has done so within 48 hours, whereas most of the fluorescein from the explant enters after 48 hours. Taken together, these data indicate that compared with periocular injection of 5 mg of fluorescein in a viscous vehicle, the same injection into a preplaced flexible device results in a much greater percentage of the total dose entering the eye, and the entry occurs over a longer period.

Histology of eyes in which a rigid exoplant was tightly sutured for two months showed no evidence of fibrous ingrowth that might reduce transscleral diffusion (Figs. 6A, 6B). Eyes in which a flexible, refillable exoplant had been present for several months also showed no fibrous ingrowth. Since these explants were not sutured to the eye, they did not stay in place during section, but the area of the sclera to which they had been apposed could be identified and showed only loose fibrin-like material (Fig. 6C).

### DISCUSSION

Local drug delivery to the eye is an important aspect of treatment development for retinal and choroidal diseases. Repeated intraocular injections have become the default approach because they do not require new technology. However, developing new technology that allows local delivery in ways that are less invasive and provide sustained therapeutic drug levels in the retina and choroid is a high priority.

TABLE 1. Pharmacokinetics of Transscleral Delivery of Fluorescein from Indented or Apposed Rigid Episcleral Explants or Periocular Injection

	Retina			Posterior Vitreous			Plasma		
	Indented Explant	Apposed Explant	Periocular Injection	Indented Explant	Apposed Explant	Periocular Injection	Indented Explant	Apposed Explant	Periocular Injection
$C_{max}$	2,133* ± 354	1,690 ± 835	64 ± 6	448 ± 138	339 ± 76	41 ± 7	532* ± 149	520* ± 60	1,454 ± 355
$AUC_{0-48h}$	16,528* ± 1,771	9,840 ± 4,173	722 ± 181	3,796 ± 1,020	3,020 ± 514	570 ± 51	—	—	—
$AUC_{0-last}$	18,076* ± 2,005	10,659 ± 4,467	1,256 ± 461	4,234 ± 1,217	3,246 ± 616	660 ± 39	3,780 ± 461	4,090 ± 702	3,663 ± 744
$AUC_{0-infinity}$	18,310* ± 1,961	10,918 ± 4,460	1,892 ± 951	4,338 ± 1,284	3,277 ± 627	705 ± 43	—	—	—
$T_{max}$ (h)	—	—	—	—	—	—	4.0 ± 1.0	2.0 ± 0.6	0.3 ± 0.0

The maximum concentration of fluorescein ( $C_{max}$ ) achieved during the experiment (ng/mL ± SE) and the area under the fluorescein concentration-time curve for the first 48 hours ( $AUC_{0-48h}$ ), for the entire duration of the experiment from the first to last time point ( $AUC_{0-last}$ ), and from the first time point extrapolated to infinity ( $AUC_{0-infinity}$ ) were computed for the retina and posterior vitreous, after placement of indented or apposed explants, or periocular injection. For plasma,  $C_{max}$ ,  $AUC_{0-48h}$ , and the time until  $C_{max}$  was reached were computed.

\*  $P < 0.05$  for difference from periocular injection by ANOVA with Bonferroni correction for multiple comparisons.

Injection of drugs into the periocular space is appealing, because it is easy and safe. In vitro studies have demonstrated that the sclera is a highly porous tissue that is permeable to large molecules.<sup>12,15,16</sup> Transscleral delivery of large molecules also appears achievable in vivo, because delivery of FITC-labeled IgG to the periocular space of rabbits at a rate of 2.5  $\mu$ g/h using an osmotic pump resulted in substantial fluorescence in the choroid and retina at several time points over a 28-day period.<sup>17</sup> However, another study has demonstrated that even for small molecules under some conditions transscleral penetration can be problematic, because only 0.12% of gadolinium-DTPA (Gd-DTPA) contained in a polyvinyl alcohol polymer disc placed at the equator of a rabbit eye was detectable in the vitreous after 3 hours.<sup>18</sup> If rabbits were euthanized immediately after implantation, levels of Gd-DTPA in the vitreous 3 hours later were 30 times higher than those in living rabbits. A subsequent study has suggested that a major barrier to transscleral drug delivery in the living rabbit is absorption of drug by periocular lymphatics and blood vessels and that this barrier can be circumvented by increasing the dose of drug delivered to the periocular space.<sup>19</sup> This may explain why continuous delivery of high levels of IgG to the periocular space results in detectable intraocular levels<sup>17</sup> and why expression of antiangiogenic proteins in the periocular space by gene transfer results in measurable levels in the choroid that suppress choroidal neovascularization.<sup>20-22</sup>

In this study, we explored an alternative to delivery of high drug levels to the periocular space to overcome loss to the systemic circulation. We have demonstrated that sealing an impermeable device containing an agent to the sclera results in selective diffusion through the sclera with minimal absorption by blood vessels in the periocular space. Two prototypes for this approach have been developed, one that would be most applicable for use with a solid sustained release formulation, such as a polymer, and a second consisting of a refillable explant that would allow replenishment of drug within the device using liquid formulations or suspensions containing microspheres that release drug slowly over time. Both approaches require the same surgical techniques that are currently used for scleral buckling surgery for which retinal surgeons have high proficiency.

Sodium fluorescein, a small molecule that is easily detected and quantified at precise locations within the eye by ocular fluorophotometry was used to test the concept. This approach allowed sequential measurements in living animals providing an outstanding assessment of fluorescein distribution within the eye over time. Unlike in vitro studies of scleral penetration that neglect the potential impact of choroidal blood flow on elimination of drug, our measurements were made in eyes with normal choroidal circulation and therefore are more likely to be predictive of transscleral drug delivery in patients. One limitation is that rabbit sclera is thinner than human sclera. The thicker sclera in humans could slow the process somewhat, but there is no reason to believe it would substantially change it. Although the resolution of fluorophotometry allows measurement of fluorescein levels at many locations in the vitreous cavity and anterior chamber, it does not allow measurements at different locations in the retina, choroid, and sclera. Such measurements cannot be performed in vivo and can only be achieved at a single time point in each animal by precise microdissections and sensitive assays.<sup>20-22</sup> Regardless, the measurement of the amount of fluorescent compound that is able to penetrate the sclera and enter the vitreous cavity over time is extremely valuable. Since proteins and other compounds of different sizes and characteristics can be fluorescently labeled, the applications of the technique described in this study are enormous.

TABLE 2. Pharmacokinetics of Transscleral Delivery of Fluorescein from Flexible Episcleral Explants or Periocular Injection

	Retina		Posterior Vitreous		Plasma	
	Explant	Injection	Explant	Injection	Explant	Injection
$C_{\max}$	3,127* $\pm$ 306	1,168 $\pm$ 178	931* $\pm$ 177	190 $\pm$ 59	237* $\pm$ 61	1,517 $\pm$ 214
$AUC_{0-48h}$	53,297* $\pm$ 7,624	3,499 $\pm$ 334	16,755* $\pm$ 1,446	893 $\pm$ 238	—	—
$AUC_{0-last}$	144,381* $\pm$ 15,057	3,876 $\pm$ 328	43,589* $\pm$ 7,121	994 $\pm$ 255	6,139 $\pm$ 2,081	4,635 $\pm$ 1,629
$AUC_{0-infinity}$	178,866* $\pm$ 34,169	3,927 $\pm$ 305	50,514* $\pm$ 10,055	1,006 $\pm$ 255	—	—
$T_{\max}$	—	—	—	—	2.5*	1.0

The maximum concentration of fluorescein ( $C_{\max}$ ) achieved during the experiment (ng/mL  $\pm$  SE) and the area under the fluorescein concentration-time curve for the first 48 hours ( $AUC_{0-48h}$ ), for the entire duration of the experiment from the first to last time point ( $AUC_{0-last}$ ), and from the first time point extrapolated to infinity ( $AUC_{0-infinity}$ ) were computed for the retina and posterior vitreous after placement of a flexible explant or periocular injection. For plasma,  $C_{\max}$ ,  $AUC_{0-ast}$ , and the time until  $C_{\max}$  was reached were computed.

\*  $P < 0.05$  for the difference from periocular injection by ANOVA with Bonferroni correction for multiple comparisons.

We used the ocular fluorophotometry technique to perform comparisons between periocular injections of fluorescein compared to injections into an impermeable device secured to the sclera. Sequential scans after periocular injection of fluorescein showed a major early peak of fluorescein in the anterior chamber with increasing levels of fluorescein in the vitreous at subsequent time points, suggesting that a significant amount of fluorescein injected into the periocular space diffuses anteriorly and enters the eye through the cornea. When fluorescein is placed in a device that is tightly sutured to create a seal with the sclera, sequential ocular fluorophotometry scans show a different profile than those after periocular injection. There is an early peak in the retina and posterior vitreous with increasing levels of fluorescein in the anterior part of the eye at later time points, indicating posterior entry through the sclera and a posterior to anterior concentration gradient within the eye. When fluorescein is placed in a device that is sutured so that there is apposition, but not a tight seal with the sclera, two early peaks occur, one in the retina and one anteriorly. Over time, the peaks come together and merge, suggesting substantial entry through the sclera and the cornea, which suggests that establishment of a scleral seal with an impermeable device promotes transscleral delivery of drug included within the explant and reduces its spread around the eye to the cornea. It is also possible that scleral defects and/or distortion caused by the sutures enhances transscleral diffusion, but this would apply only to the apposed explant group, because the sutures are outside the edges of the device, and very little lateral diffusion outside the device occurred in the indented group.

The flexible silicone refillable device provides an opportunity for direct comparison of periocular injection of fluorescein with injection into a preplaced device. The compressible nature of the silicone device facilitates the creation of a scleral seal, which is maintained without indentation of the sclera. Pharmacokinetic analysis showed that peak levels of fluorescein in the retina and posterior vitreous were significantly increased by injection of fluorescein into the device compared with its injection into the periocular space. Also, a greater percentage of the fluorescein from the device is able to access the eye, resulting in exposure over time that is 37-fold higher in the retina and 44-fold higher in the posterior vitreous than the exposure achieved with periocular injection. Fluorescein levels in plasma showed a significantly higher peak after periocular injection than that seen after injection into the explant, confirming that after periocular injection, a large percentage of the injected dose is absorbed into the systemic circulation, thereby bypassing the eye and reducing intraocular levels. Achieving a fairly tight seal between the sclera and the edges of the device enhances transscleral delivery. Indented rigid devices resulted in higher peak levels of fluorescein in the retina than did periocular injection, whereas this was not the case

with apposed devices that were not sealed. The seal prevents tangential diffusion of drug and absorption by periocular blood vessels and also limits ingrowth of fibrous tissue into the explant, which can cover the sclera and reduce its permeability. It is possible that fibrous tissue will grow over the refillable device, making it difficult to identify the port for repeated injections; long-term studies are needed to determine whether this is the case.

Our results are consistent with those of a previous study that demonstrated transscleral delivery of betamethasone or 6-carboxy fluorescein diacetate from an episcleral explant in rabbits.<sup>23</sup> The explant used in that study was also designed to limit diffusion of drug away from the sclera but, in addition, drugs were incorporated into a polymer that provided sustained release. Experiments with 6-carboxy fluorescein diacetate showed fluorescence that was highest in the retina and choroid beneath the explant and decreased along a fairly steep spatial gradient extending away from the explant, and experiments with betamethasone demonstrated maintenance of pharmacologically relevant levels in the retina and choroid for at least 4 weeks, the longest time point examined. Together, these studies demonstrate that transscleral delivery of drugs combined with sustained-release strategies is potentially advantageous for treatment of retinal and choroidal diseases. Additional studies are needed to optimize sustained delivery and investigate specific therapeutic agents, to try to translate these findings into practical benefits for patients.

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