Measurement and Prediction of Lateral Diffusion within Human Sclera

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PURPOSE. Drug delivery via the sclera is a promising approach to retinal disorder treatments that require access to the posterior segment of the eye. To complement existing studies of transverse diffusion across the sclera, this study examined lateral diffusion within the sclera parallel to the scleral surface.

METHODS. Using sulforhodamine as a model hydrophilic drug, rates of diffusion were measured in strips of human cadaveric sclera for up to 1 week. Data were analyzed with a mathematical model based on theoretical expressions for one-dimensional diffusion.

RESULTS. Measurable amounts of sulforhodamine were detected at distances of 5 and 10 mm from the sulforhodamine donor reservoir at 4 hours and 3 days, respectively. The effective lateral diffusivity of sulforhodamine was determined to be $3.82 \times 10^{-6}$ cm$^2$/s, which is similar in magnitude to the transverse diffusivity. The theoretical model agreed with experimental values with an average error of 39%.

CONCLUSIONS. This study shows that the lateral diffusion of sulforhodamine in human sclera is slow and localizes to the site of administration. (Invest Ophthalmol Vis Sci. 2006;47:3011–3016) DOI:10.1167/iovs.05-1464

Targeted administration of drugs to the posterior segment of the eye remains a significant challenge in ocular drug delivery. Current treatment strategies include systemic delivery, by oral or parenteral routes, and local delivery using topical drops, subconjunctival and peribulbar injections, intravitreal injections, and implants.1,2 However, none of these approaches provides fully satisfactory ocular delivery to the posterior part of the eye.

Systemic delivery is often accompanied by side effects because of the high drug doses needed to reach the target tissues within the eye. Topical drops through the cornea generally cannot achieve adequate drug concentrations in the posterior segment due to slow diffusion across the cornea to the back of the eye and counterproductive convection of tear fluid and aqueous humor.3-5 Although intraocular injection and implants can provide delivery targeted to the posterior segment, they can lead to complications such as retinal detachment, hemorrhage, endophthalmitis, and cataract, especially when repeated injections are required.3,4

Because of these limitations, there is growing interest in drug delivery across the sclera, which avoids the complications associated with penetrating the globe and the diffusion barrier of the cornea.5 The sclera’s large surface area, which averages 17 cm$^2$ on the human eye,5 is approximately 20 times larger than the cornea. Moreover, sclera is much more permeable, especially to large and hydrophilic drugs.5,6 Conventional subconjunctival and peribulbar injections provide access to the transscleral route. Novel delivery systems, involving implants, gels, and patches applied to the scleral surface, and intrascleral injections are being developed to enable extended-release and better-targeted drug delivery via the sclera.2

Motivated by these opportunities, a number of studies have examined rates of diffusion across the sclera as a function of molecular size and other parameters.5-12 However, little attention has been given to diffusion within the sclera in the lateral direction parallel to the scleral surface, which could affect drug distribution caused by lateral spread of the released drug—for example, from an extraocular implant or intrascleral injection. The nonisotropic architecture of collagen lamellae and other features of scleral microanatomy1,5 suggest that lateral diffusion may behave differently from transscleral diffusion.

This study presents the first experimental measurements of lateral diffusion within the sclera, with sulforhodamine used as the model drug, and provides a theoretical model that predicts the diffusion profile as a function of both time and distance along the sclera. Lateral diffusivity is also compared to transverse diffusivity across the sclera to identify possible differences.

EXPERIMENTAL AND THEORETICAL METHODS

Lateral Diffusion Measurements

The lateral diffusion profile of a model drug, sulforhodamine (558 Da; Invitrogen, Eugene, OR), was measured through human cadaveric sclera using spectrophotometry. Human sclera was obtained from the Georgia Eye Bank (Atlanta, GA) and stored in a moist container for 2 to 5 days at 4°C. Adherent tissues associated with the retina, choroid, and episclera were gently removed with cotton swabs. Swabs of full-thickness sclera measuring 10 to 15 mm in length and 3 to 5 mm in width were cut from the globes using surgical scissors and razor blades.

In this study, we used human cadaveric sclera, which has the advantage of being human tissue and enabling better control over the experimental system through the in vitro environment. Although an in vivo animal study would provide an improved physiological environment, this study focused on the process of diffusion within the sclera, which is governed largely by the nonliving collagen and extracellular matrix structures and does not address the effects of, for example, blood flow or other active processes.

A glass vial was filled with 1 mL of donor solution containing $9.0 \times 10^{-5} \text{ M}$ sulforhodamine in physiological saline (Balanced Salt Solution [BSS]; Alcon Laboratories, Fort Worth, TX). A scleral strip was suspended vertically in the glass vial such that the lower 3 mm of the tissue dipped into the donor solution (Fig. 1). The vial was then capped and sealed with parafilm to maintain scleral hydration and placed in a 37°C water bath, although some tissue dehydration probably still

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Transscleral Diffusion Measurements

Sulforhodamine diffusion was also measured across the sclera with a flow-through permeation chamber. According to the procedure described previously,14 scleral discs 10 to 15 mm in diameter were excised from human globes and mounted in two-compartment perfusion chambers. A 300-μL depot of 9.0 × 10^{-5} M sulforhodamine donor solution was added to the episcleral surface while BSS was perfused across the choroidal side. Every 1 hour, a fraction containing 2 mL of the perfusate was collected over a 24-hour period and its fluorescence concentration was measured by spectrofluorimetry. From these measurements, the effective transverse diffusivity ($D_{\text{trans}}$) was calculated as

$$D_{\text{trans}} = \frac{C_{\text{cuvette}} \cdot V_{\text{cuvette}} \cdot d}{A \cdot \Delta t}, \quad (2)$$

where $d$ is scleral thickness (0.6 mm), $A$ is the scleral surface area exposed to donor solution (0.37 cm²), $\Delta t$ is sampling time (1 hour) and $C_{\text{donor}}$ is the sulforhodamine concentration in the donor solution, which was initially at 9.0 × 10^{-5} M and decreased over time. This effect was accounted for by correcting $C_{\text{donor}}$ in the calculation by using equation 2. The transscleral permeability coefficient can be obtained by dividing the transverse diffusivity by scleral thickness.15

Sclera-to-Saline Distribution Coefficient

An additional experiment was performed to determine the sclera-to-saline distribution coefficient to examine possible binding between sulforhodamine and scleral tissue. A full-thickness scleral strip was submerged in a 9.0 × 10^{-5} M sulforhodamine donor solution for 24 hours. After the tissue was removed and rinsed with the saline solution, it was sectioned into 50-μm-thick pieces on a cryostat. All tissue pieces were collected and incubated in 20 mL of BSS. The sulforhodamine concentration in the solution was measured over time by spectrofluorimetry until it reached a constant value (after ~1 hour). Assuming 100% sulforhodamine extraction efficiency, the sclera-to-saline distribution coefficient ($K_D$) was obtained as

$$K_D = \frac{C_{\text{sclera}}}{C_{\text{bath}}}, \quad (3)$$

where $C_{\text{sclera}}$ is the concentration in the sclera determined by extraction, and $C_{\text{bath}}$ is the original concentration of the donor solution (9.0 × 10^{-5} M).

Theoretical Model

A theoretical model was developed to predict the concentration of sulforhodamine as a function of both time and distance during lateral diffusion within human sclera. A one-dimensional model was justified by the geometry of the experimental setup, which was symmetric in both of the horizontal dimensions of the scleral strip and provided a concentration-gradient driving force for diffusion only in the vertical direction (Fig. 1). We further recognized that the sulforhodamine could be present within the sclera in two forms: free sulforhodamine that can diffuse and bound sulforhodamine immobilized at binding sites in the scleral tissue. Previous studies have suggested that compounds structurally similar to sulforhodamine bind within sclera.8

Transient, one-dimensional diffusion with binding in a semi-infinite slab can be modeled mathematically as16

$$\frac{dC_{\text{free}}}{dt} = \frac{D_{\text{eff}}}{\Delta x^2} \left( k_1(C_{\text{free}} - K_{\text{eq}}C_{\text{bound}}) \right) \quad (4)$$

and

$$\frac{dC_{\text{bound}}}{dt} = k_1(C_{\text{free}} - K_{\text{eq}}C_{\text{bound}}). \quad (5)$$

where $C_{\text{free}}$ is the free sulforhodamine concentration in the sclera, $C_{\text{bound}}$ is the bound sulforhodamine concentration in the sclera, $k_1$ is the binding rate constant, $D_{\text{eff}}$ is the effective lateral diffusivity of free sulforhodamine in the sclera, $x$ is the lateral position in the sclera, and $K_{\text{eq}}$ is the ratio of free-to-bound sulforhodamine at equilibrium in the sclera.
For the evaluation of bound versus free sulforhodamine in the sclera, the sclera-to-saline distribution coefficient (equation 3) can be re-expressed as

\[ K_\text{d} = \frac{C_{\text{free}}}{C_{\text{bath}}} = \frac{C_{\text{free}} + C_{\text{bound}}}{C_{\text{bath}}} \]  

(7)

Because the liquid portion of the sclera is composed of saline of similar composition to that of the surrounding bath, we can assume \( C_{\text{free}} \) is equal to \( C_{\text{bath}} \). Thus,

\[ K_\text{d} = 1 + \frac{C_{\text{bound}}}{C_{\text{free}}} = 1 + \frac{1}{K_{\text{eq}}} \]  

(8)

Using equation 8, as well as the assumption that sulforhodamine binding in the sclera is at equilibrium (\( C_{\text{free}} = K_{\text{eq}}C_{\text{bath}} \)), Equations 4 and 5 can be rewritten as

\[ \frac{d}{dt} \left( \frac{C_{\text{free}} + C_{\text{bath}}}{K_{\text{eq}}} \right) = D_{\text{lat}} \frac{d^2 C_{\text{free}}}{dx^2} \]  

(9)

Rearranging equation 9 yields the following expression

\[ \frac{dC_{\text{free}}}{dt} = D_{\text{lat}} K_{\text{eq}} \frac{d^2 C_{\text{free}}}{dx^2} \]  

(10)

In solving equation 10, the initial and boundary conditions are

\[ C_{\text{free}}(x,0) = 0, \]
\[ C_{\text{free}}(0,t) = C_{\text{donor}} K_{\text{d}}, \] and
\[ C_{\text{free}}(z,t) = 0, \]

where \( z \) is the length of the scleral strip above the donor solution. Solving equation 10 subject to the conditions in equation 11 yields the final expression for sulforhodamine concentration in the sclera as a function of time and position:

\[ C_{\text{free}}(x,t) = C_{\text{donor}} K_{\text{d}} \left( 1 - \operatorname{erf} \left( \frac{x}{2 \sqrt{D_{\text{lat}} t}} \frac{K_{\text{eq}}}{1 + K_{\text{eq}}} \right) \right) \]  

(12)

where \( \operatorname{erf} \) is the error function.

### Mean Absolute Percent Error

Mean absolute percent error (MAPE) was used for statistical analysis of the difference between theoretical predictions and experimental data. MAPE is calculated by averaging the percentage difference between predicted values and experimental data:

\[ \text{MAPE} = \frac{1}{n} \sum \left| \frac{C_{\exp} - C_{\text{theor}}}{C_{\exp}} \right| \times 100\% \]  

(13)

where \( C_{\exp} \) is the experimentally measured concentration, \( C_{\text{theor}} \) is the theoretically predicted concentration, and \( n \) is the number of measurements.

### Results

In this study, we sought to image and quantify lateral diffusion within the sclera and to compare lateral and transverse diffusivity. A series of experiments were conducted to measure the sulforhodamine concentration profile within human cadaveric sclera at several time points between 4 hours and 1 week. A mathematical model of one-dimensional, semi-infinite diffusion was also developed to predict the diffusion profiles and compare with the experimental data.

### Imaging Lateral Diffusion within the Sclera

An initial experiment was performed to provide visual images showing the progression of sulforhodamine diffusion along the sclera as a function of time and position. Figure 2 shows representative cross-sectional views of sclera frozen after 24 hours of sulforhodamine diffusion and then sectioned for viewing by fluorescence microscopy. Within each scleral slice, the sulforhodamine concentration appears uniform, which indicates that vertical diffusion occurred at the same rate, independent of position in the horizontal direction. This observation is consistent with modeling sulforhodamine diffusion as a one-dimensional process. Scleral sections collected further from the sulforhodamine donor solution show progressively lower sulforhodamine concentrations over the ~1-cm scleral strip.

### Quantifying Lateral Diffusion within the Sclera

To quantify the lateral diffusion profile within the sclera, sulforhodamine concentration was measured in the scleral sections as a function of both time and distance along the sclera. Figure 3 shows the resultant concentration profiles over a distance of 11 mm along the sclera at time points between 4 hours and 1 week (i.e., 168 hours). After 4 hours, sulforhodamine diffusion was detected at a distance up to 5 mm along the sclera. After 1 week, sulforhodamine diffused farther than 1 cm along the sclera. At each time point, concentration decreased with increasing distance (analysis of variance (ANOVA), \( P < 0.0001 \)). Over time, the sulforhodamine concentration at each position increased with time (ANOVA, \( P < 0.0001 \)).

### Determining Lateral Diffusivity

To determine the effective lateral scleral diffusivity of sulforhodamine from the data in Figure 3, we used a theoretical model of one-dimensional diffusion (equation 12). As parameters for this model, we measured the sclera-to-saline distribution coefficient (\( K_{\text{eq}} \)) experimentally to be 13.6, which indicates a strong binding between sulforhodamine molecules and the sclera tissue. The free-to-bound sulforhodamine ratio (\( K_{\text{eq}} \)) was then calculated with equation 8 to be 0.08. This left sulforhodamine diffusivity (\( D_{\text{lat}} \)) as the only unknown variable.

The diffusion model was then fitted to the experimental data, as shown in Figure 4, which yielded an effective sulforhodamine diffusivity of \( D_{\text{lat}} = 3.82 \times 10^{-6} \text{ cm}^2/\text{s} \).

Visually, the predicted curves in Figure 4 capture the trend of the data, but show some disagreement. The quality of this fit can be gauged quantitatively by its MAPE of 39%, which indicated that predicted values were on average within 39% of experimental values. This uncertainty can be compared to the average standard error associated with experimental measurement (i.e., the average of error bars in Fig. 3), which was calculated to be 60%. Thus, the error associated with the experimental measurements is greater than the disagreement between the theoretical model and the experimental data, which means that the theoretical model predictions are as good as possible, given the uncertainty in the data.

Further examination shows that at early times (e.g., 4 hours), the model generally underpredicted the data, whereas
at later times, it generally overpredicted the data. This finding can be explained by a changing diffusivity, which was initially larger than the overall fitted value and later was smaller. Diffusivity may have changed over time because of changes in tissue hydration. Although the sclera was maintained in a humid environment, some tissue dehydration could have occurred over the course of the 1-week experiment. Decreasing tissue hydration could progressively decrease diffusivity in the sclera as the aqueous diffusion pathways decrease in number and size. In addition, decreased water content of the sclera could also decrease average tissue sulforhodamine concentrations by decreasing the aqueous regions containing sulforhodamine relative to the collagen, GAG, and other insoluble regions.

Comparing Lateral and Transverse Diffusivities

To compare lateral and transverse diffusion in the sclera, we measured the rate of transverse diffusion of sulforhodamine across the sclera, which provided an effective diffusivity of $D_{\text{trans}} = 1.28 \pm 0.22 \times 10^{-6} \, \text{cm}^2/\text{s}$, which corresponds to a permeability of $2.15 \pm 0.37 \times 10^{-5} \, \text{cm/s}$. These values compare well with previously reported experimental data for scleral permeability of other molecules of similar molecular weight and to a predicted diffusivity of $2.5 \times 10^{-6} \, \text{cm}^2/\text{s}$ for sulforhodamine generated using an independent theoretical model described previously (calculation not shown). Comparing the lateral ($D_{\text{lat}} = 3.82 \times 10^{-6} \, \text{cm}^2/\text{s}$) and transverse ($D_{\text{trans}} = 1.28 \times 10^{-6} \, \text{cm}^2/\text{s}$) diffusivity values generated in this study indicates that diffusing in the lateral
This study provides the first measurements of lateral diffusion within human cadaveric sclera. Measurable amounts of drug were detected at distances of 5 and 10 mm from the drug donor reservoir at 4 hours and 3 days, respectively. Experimental data were used to calculate an effective lateral diffusivity of $3.82 \times 10^{-6}$ cm$^2$/s. This calculation enabled the prediction that a point source of sulforhodamine would require 6 weeks to diffuse through the sclera. However, based on a similar calculation, it should take at least 6 weeks for sulforhodamine to diffuse from a localized source throughout all the sclera in a human eye (using a characteristic distance of 3.75 cm, which is half the circumference of the human eye$^{13}$).

Although validated using only one compound, the model developed in this study for scleral diffusion is general. It should be valid for both hydrophilic and lipophilic compounds, as well as small drugs and macromolecules, given knowledge of their effective diffusivity, distribution coefficient, and binding constant in the sclera, as shown in equation 12. Effective diffusivity should be strongly reduced by increases in molecular size, but only weakly affected by lipophilicity. Binding constant and distribution coefficient (which is strongly influenced by the binding constant) should be strongly influenced by molecular properties, such as lipophilicity.

Measurements and calculations of lateral diffusion in this study have assumed that diffusion is one-dimensional and no drug exits the sclera along its choroidal or episcleral surfaces. This is desirable—and it naturally would be true for drug delivery localizations on the millimeter scale for hours to days. Conversely, if lateral distribution of drug over a larger area with faster kinetics is required, then a less localized injection or implant that covers a larger area of scleral surface may be needed. Moreover, drug distribution by the vasculature in the choroid has not been considered in this analysis and may provide a means for additional drug distribution over larger areas.

**CONCLUSIONS**

Lateral diffusion of sulforhodamine, a hydrophilic model drug, was studied in human cadaveric sclera. Measurable amounts of drug were detected at distances of 5 and 10 mm from the drug donor reservoir at 4 hours and 3 days, respectively. Experimental data were used to calculate an effective lateral diffusivity of $3.82 \times 10^{-6}$ cm$^2$/s. This calculation enabled the prediction that a point source of sulforhodamine would require 6 weeks to diffuse throughout all the sclera in a human eye. Comparison of experimental measurements of lateral diffusion within the sclera to transverse diffusion across the sclera indicated similar effective diffusivities, although lateral diffusion was approximately three times faster. A theoretical model for one-dimensional diffusion in the sclera was developed and shown to be valid for both hydrophilic and lipophilic compounds, as well as small drugs and macromolecules, given knowledge of their effective diffusivity, distribution coefficient, and binding constant in the sclera.

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