

Depth-resolved monitoring of glucose diffusion in tissues by using optical coherence tomography

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We demonstrate the capability of the optical coherence tomography (OCT) technique for depth-resolved monitoring and quantifying of glucose diffusion in fibrous tissues (sclera). The depth-resolved and average permeability coefficients of glucose were calculated. We found that the glucose diffusion rate is not uniform throughout the tissue and is increased from approximately $2.39 \pm 0.73 \times 10^{-6}$ cm/s at the epithelial side to $8.63 \pm 0.27 \times 10^{-6}$ cm/s close to the endothelial side of the sclera. Results demonstrated that the OCT technique is capable of depth-resolved monitoring and quantification of glucose diffusion in sclera with a resolution of approximately 40 μ m. © 2006 Optical Society of America
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Functional imaging, monitoring, and quantification of diffusion processes in epithelial and connective tissues *in vivo* are extremely important for many biomedical applications, including therapy and diagnostics of various diseases. Several pathological and disease conditions might alter physical and physiological properties of the extracellular matrix. Changes in the diffusion constants of certain chemical compounds could be used as a diagnostic tool for differentiation of healthy and abnormal tissues and could thus be utilized for early diagnostics. Additionally, selecting the translucence of the upper tissue layers by reducing the tissue's scattering coefficient is a key technique for structural and functional imaging, particularly for detecting local static or dynamic inhomogeneities hidden by a highly scattering medium.¹ Development of a practical solution for monitoring and quantifying the molecular diffusion in tissue layers (with distinct morphological and cellular structures and, hence, different diffusion constants) *in vivo* will help development of novel therapeutic agents, drug delivery techniques, and novel clinical diagnostic methods.

In the past decades, several scientific groups have been developing and applying different experimental techniques to study molecular diffusion in tissues *in vitro*, including spectrophotometry, spectrofluorometry, fluorescence microscopy, and microdialysis.^{1,2} These techniques to date had supplied our knowledge about drug diffusion in different tissues from *in vitro* and a few *in vivo* studies.

In this Letter we report results of pilot studies on application of optical coherence tomography³ (OCT) for depth-resolved monitoring and quantifying of glucose diffusion in rabbit sclera *in vitro*. The sclera has a large and accessible area, a high degree of hydration, and has a permeability that does not noticeably

change with age.⁴ The sclera is composed of three main layers: the episclera, the stroma, and the lamina fusca. The size, distribution, orientation, and packing of collagen bundles in these layers are different.⁵

A major source of scattering in tissues is the refractive index mismatch among the cellular contents, structural components (such as collagen and elastin fibers), and extracellular fluid. The increase of tissue analyte concentration will raise the refractive index of the extracellular fluid, reduce the refractive index mismatch, and thus decrease the scattering coefficient of the tissue as a whole. Another possible mechanism for a chemical compound to change the tissue scattering coefficient is by changing the local concentration of scattering particles as a result of tissue dehydration caused by hyperosmolarity of the chemical agent. Changes in the volume fraction of tissue components will change the scatterer packing parameter and therefore will change the scattering coefficient. Since the OCT technique measures the in-depth light distribution, changes in the in-depth distribution of the tissue scattering coefficient of refractive index are reflected in changes in the OCT signal.⁶ Thus, because the diffusion of chemical compounds in tissues introduces local changes in their optical properties, one can monitor and quantify the diffusion process by analyzing the amplitudes and exponential profile of light attenuation in tissue with the OCT technique.^{1,6,7}

A time-domain OCT system was utilized in these experiments. The optical source used in this system is a low-coherence broadband light source (Superlum, Inc., Russia) with a wavelength of 1310 ± 15 nm, output power of 375 μ W, and resolution of 25 μ m (in air). Light in the sample arm of the interferometer was directed into the tissues through a miniature en-

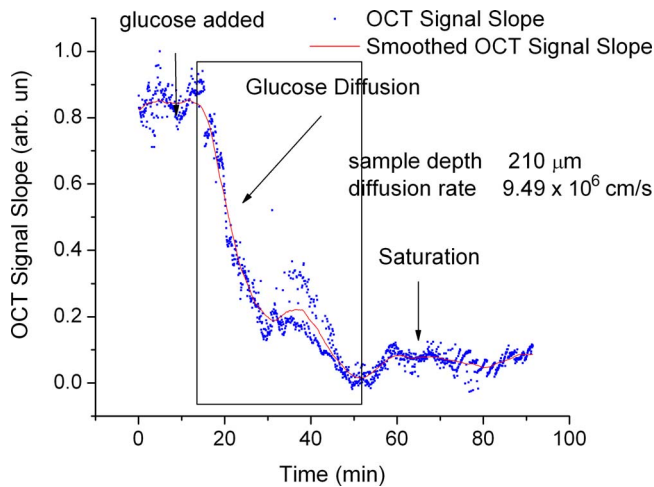


Fig. 1. (Color online) OCT signal slope as a function of time recorded from sclera during the glucose diffusion experiment. The box denotes the times of glucose diffusion in the selected region.

oscopic probe (Imalux Corporation, Cleveland, Ohio). This probe allowed lateral scanning of the sample surface in the lateral direction (x axis). In-depth scanning (z axis) was produced electronically by piezoelectric modulation of the fiber length. The acquired images were $2.2 \text{ mm} \times 2.4 \text{ mm}$. The images were continuously acquired for 90–150 min during experiments at the rate of 3 s per image. The 2D images were averaged in the lateral direction (over $\approx 1 \text{ mm}$ at the most linear part of the OCT image) into a single curve to obtain an OCT signal that represents the 1D distribution of light in depth on a logarithmic scale.

Experiments were performed by using fresh rabbit eyes. The eyes were kept cooled in a 0.9% NaCl solution (pH 5.5) during transportation and storage. Experiments were conducted in the first 3 days upon enucleating to ensure no change in the physiological status of the tissues. (OCT analysis revealed no noticeable changes in the first 3 days; however, after 4–5 days, scleral samples were enlarged, less scattering, and some of their connective tissues were dissociated; data not shown.) An hour before the experiments, a whole eyeball was placed in a specially designed dish containing 4 ml of saline at room temperature. The eyeballs were fully submerged in the saline during the experiments. Experiments were performed at 22°C , and a constant temperature was maintained for the duration of the trials. A laser beam was directed perpendicular to the scleral epithelial surface. The glucose solution was prepared by adding 4 ml of a 40% glucose solution to the sample in saline after 10 min from the starting point. The final glucose concentration in saline was 20% in all experiments. Sixteen rabbit eyeballs were studied.

The permeability coefficient of glucose in sclera was calculated by using two different methods: OCT signal slope (OCTSS) and amplitude (OCTA) methods. The OCTSS method was used to calculate the average permeability coefficient, \bar{P}_G , of scleral stroma. The \bar{P}_G was computed by dividing the thick-

ness of the region used to calculate the OCTSS (typically, around $155\text{--}255 \mu\text{m}$) by the time of glucose diffusion inside this region, $\bar{P}_G = z_{\text{region}} / t_{\text{region}}$. Since the diffusion of glucose reflected in changes of the OCTSS and the signals was relatively constant before application of glucose and after saturation (Fig. 1), t_{region} was calculated as the time when the saturation stage was reached minus the time when the OCTSS started to change (highlighted by a box in Fig. 1).

The OCTA method of measurement was used to calculate the permeability coefficient at the specific depths in the sclera as $P_G(z) = z_i / t_{z_i}$, where z_i is the depth at which measurements were performed (calculated from the front surface) and t_{z_i} is the time of glucose diffusion to this depth. The t_{z_i} was calculated from the time glucose was added to the tissue until glucose-induced change in the OCT amplitude commenced. Note that, unlike in the OCTSS method, z_i was calculated from the epithelial surface of the sclera to the specific depth in scleral stroma.

A typical OCTSS versus time plot recorded from sclera during the glucose diffusion experiment is shown in Fig. 1. The propagation of glucose inside the sclera changed the local scattering coefficient, which was reflected in the OCTSS. The increase in local in-depth glucose concentration resulted in the decrease of the OCTSS during the diffusion process. The overall thickness of the sclera in this experiment was $349 \mu\text{m}$ (assuming the refractive index to be equal to 1.4). The thickness of the region used for calculation was $210 \mu\text{m}$, approximately $36 \mu\text{m}$ away from the epithelial surface. The \bar{P}_G calculated from data presented in Fig. 1 was found to be $9.49 \times 10^{-6} \text{ cm/s}$. In these calculations we assumed the refractive index to be constant. However, the refractive index changes because of glucose diffusion and local tissue dehydration, and thus the physical thickness of the region used for calculation may change. Therefore addi-

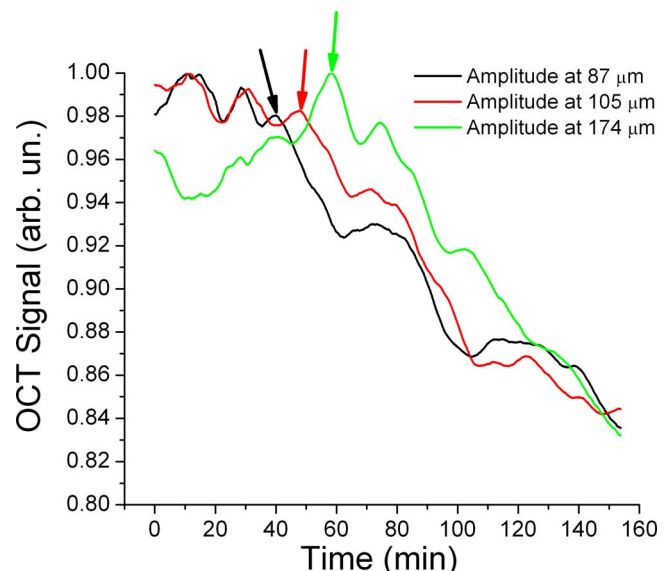


Fig. 2. (Color online) OCT signal as a function of time recorded at different depths. The arrows denote the starting point of diffusion at the particular depth in the sclera.

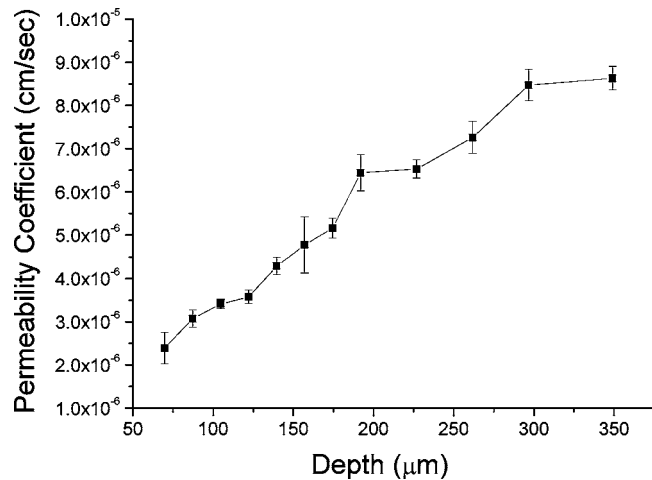


Fig. 3. Diffusion rates measured at different depths in the sclera. The average standard deviation for the permeability coefficients was $\pm 0.29 \times 10^{-6}$ and was calculated from three or four independent experiments.

tional algorithms should be utilized for precise estimations of the diffusion coefficient (e.g., as described in Ref. 8).

High in-depth resolution of the OCT technique allowed calculation of glucose diffusion rates at different depths of the tissue samples. Figure 2 shows typical OCT signals measured at different depths during glucose diffusion experiments. This figure demonstrates the time delay of the glucose front reaching different tissue depths. The glucose fronts were identified by depth-resolved monitoring of progression of glucose-induced changes in the OCTA (diffusion pattern) with time.

Figure 3 shows typical permeability coefficients of glucose measured at different depths in a sclera by using the OCTA method. Figure 3 shows that the glucose diffusion rate inside the sclera is nonlinear and is increased from $2.39 \pm 0.73 \times 10^{-6}$ cm/s at the epithelial side to $8.63 \pm 0.27 \times 10^{-6}$ cm/s close to the endothelial side of the sclera. This nonlinearity is due to glucose diffusion through at least two layers: epithelium (low diffusion) and stroma (faster diffusion). Different inclusions of these two processes at different time intervals relative to the calculated permeability coefficients are the source for such nonlinearity. Collagen packing and size could also contribute to the observed curve, because they may be different at different depths. Therefore Fig. 3 for the first time provides experimental demonstration of depth-resolved analysis of analyte diffusion in tissues.

In this Letter we have demonstrated the possibility of depth-resolved monitoring and quantifying of glucose diffusion in tissues. We found that the glucose permeability is nonlinear through the tissue and is increased from 1.88×10^{-6} – 2.91×10^{-6} cm/s at the epithelial side to 8.44×10^{-6} – 8.82×10^{-6} cm/s close to the endothelial side of the sclera in all experi-

ments. The average permeability coefficient of 20% concentrated glucose solution in stroma was found to be $8.87 \pm 1.4 \times 10^{-6}$ cm/s from independent experiments in 16 rabbit sclera samples. Since the experiments were performed in the whole-eye configuration, the eyes were kept as close as possible to the natural physiological status. Sclera tissues were a little overhydrated because they were fully submerged in saline during the experiments. However, since it has been previously shown that hydration plays a key role in the study of the diffusion rate,⁴ our future experiments will be conducted *in vivo* to eliminate the hydration factor.

Data received in this study for glucose permeability in rabbit sclera agree well with *in vitro* data of human eye sclera measured by using a conventional collimated light transmission technique⁹: $\bar{P}_G = 11.4 \times 10^{-6}$ cm/s.

The presented results encourage us to believe that OCT is an effective tool for studying permeability and analyte (including drug) diffusion in tissues, both in specified regions and at different tissue depths. However, the currently utilized OCT system allowed calculation of dependable permeability coefficients at the specific depths in the sclera [$P_G(z)$] in approximately 40 μm increments (see Fig. 3) owing to (1) limited resolution of the OCT system (25 μm) and (2) high speckle noise. Application of ultrahigh-resolution OCT systems, speckle-reduction algorithms, and phase-sensitive measurements may reduce the observed standard deviation of the glucose permeability coefficient. Our future studies will be focused on development of a theoretical model of analyte diffusion in different scleral compartments (based on the size, distribution, and orientation of collagen fibrils).

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