

Quantification of Molecular Diffusion in Arterial Tissues with Optical Coherence Tomography and Fluorescence Microscopy¹

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Abstract—Alternations in vascular permeability for different molecules, drugs, and contrast agents might be a significant early marker of development of various diseases such as atherosclerosis. However, up to date experimental studies of molecular diffusion across vascular wall have been limited. Recently, we demonstrated that the Optical Coherence Tomography (OCT) technique could be applied for noninvasive and nondestructive quantification of molecular diffusion in different biological tissues. However, the viability of the OCT-based assessment of molecular diffusion should be validated with established methods. This study focused on comparing molecular diffusion rates in vascular tissues measured with OCT and standard fluorescent microscopy. Noninvasive quantification of tetramethylrhodamine (fluorescent dye) permeability in porcine vascular tissues was performed using a fiber-based OCT system. Concurrently, standard histological examination of dye diffusion was performed and quantified with fluorescent microscopy. The permeability of tetramethylrhodamine was found to be $(2.08 \pm 0.31) \times 10^{-5}$ cm/s with the fluorescent technique ($n = 8$), and $(2.45 \pm 0.46) \times 10^{-5}$ cm/s with the OCT ($n = 3$). Good correlation between permeability rates measured by OCT and histology was demonstrated, suggesting that the OCT-based method could be used for accurate, nondestructive assessment of molecular diffusion in multilayered tissues.

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INTRODUCTION

Cardiovascular diseases are the number one cause of death in the United States [1, 2]. The demand for new methods of early diagnosis of this disease has been the main motive for the development of a number of methods and techniques. It has been suggested that the development of disease and/or abnormal condition of a tissue can be caused by a distortion of its anatomical structure [3]. For example, areas with atheromatous lesions have shown an increase in permeability with methylene blue [4]. Assessment of molecular diffusion in both normal and pathological tissues could be a useful method for early diagnostics. Therefore, quantifying the permeability rate of drugs and analytes can provide significant insight towards development of a diagnostic tool. The diffusivity of analytes and molecules through biological tissues has been studied with great interest for a number of years because of the valuable information it can provide regarding tissue's state, physiological properties, and pattern of drug transport.

Several techniques have been utilized in the past to study diffusion of different analytes in tissues such as MRI, ultrasound, and fluorescence microscopy. These

methods have opened up the basic understanding of molecular kinetics in biological specimens. However, several limitations (such as long signal acquisition and processing time and low resolution) ultimately restricted their wide use. A noninvasive, high resolution imaging technique is required for assessment of the molecular kinetics in biological samples. Optical coherence tomography (OCT) is an interferometric-based system that is currently being utilized in numerous biomedical applications [5, 6]. OCT measures back-reflected photons from a point of interest inside a sample that is within the coherence length of a broadband laser source. Essentially, OCT is comparable to ultrasound imaging with the exception of the use of light instead of sound waves yielding 2D and 3D images with micrometer-scale resolution at depths of up to a few millimeters [7]. Extending of OCT imaging depth by transformation of a turbid medium to a transparent one has been the subject of several studies. The most popular method is optical clearing by refractive index matching [8]. Optical clearing could be achieved by the reduction of light scattering by the addition of an analyte, referred to as clearing agent, with a higher refractive index in order to reduce refractive index mismatch between cells and extracellular fluid [9–12]. The utilization of clearing agents could also assist in

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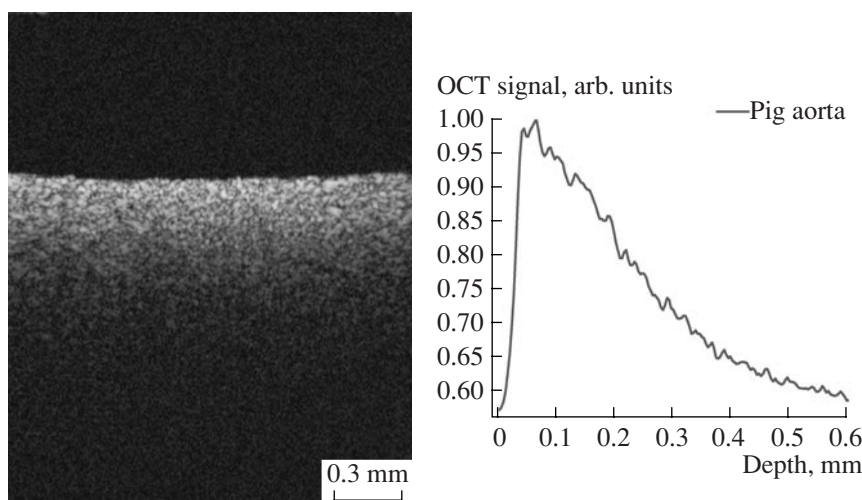


Fig. 1. (a) 2D OCT image of a pig aorta (b) and its corresponding 1D signal distribution.

improving the contrast of OCT images as well. For example, applying glycerol on skin tissues was shown to provide a better image contrast than using gold nanoparticles [13].

In our previous studies we have developed effective OCT-based methods for the determination of diffusivity of molecules and drugs by detecting changes in tissue's optical properties as the analytes perfuse through extracellular matrix [14–19]. However, in order to validate the use of OCT in noninvasive diffusion studies, a comparison with a conventional well-established method already in use had to be made. The focus of this study was to compare the permeability rate of Dextran tetramethylrhodamine fluorescent dye in vascular tissues measured with both the OCT system (completely non-destructively) and a fluorescence microscope (via standard histological examination).

MATERIALS AND METHODS

Freshly excised pig aortas (J&J Packing, Brookshire, TX) were transported to the lab while submerged in chilled 0.9% saline solution. A sagittal incision was made to the aorta creating a sheet, which was then cut into 1 cm² square samples. The permeability of Dextran tetramethylrhodamine dye (Molecular weight 3000 Daltons) (Molecular Probes, Eugene, Oregon) was monitored using two different imaging methods: OCT and the fluorescence microscope. Experiments in both imaging methods began simultaneously after the tissue had arrived. The dye solution used in the experiments was prepared by diluting 1 mg of the Dextran in 10 ml of distilled water, and was kept at 4 C and preserved from being exposed to light to prevent photo bleaching.

OCT experiments were conducted using a time-domain OCT system. The optical source was comprised of a low-coherence broadband light source uti-

lizing Near Infrared (NIR) light (Superlum, Inc., Russia) with a wavelength of 1310 ± 15 nm and an output power of 3 mW. Two-dimensional (2D) imaging was done continuously by acquiring 2.2×2.4 mm images every three seconds by laterally scanning of the sample surface with the incident beam and in-depth with the interferometer. The 2D images were then averaged laterally (over approximately 1 mm, for speckle noise suppression) to produce a single one-dimensional (1D) curve displaying in-depth light attenuation in logarithmic scale (Fig. 1).

An aorta sample was placed on top of 0.2 ml of saline solution on a glass slide in order to preserve hydration of the tissue during the course of imaging. The fluorescent dye was added about 5 min from the onset of imaging and the tissue was continuously imaged for 30 min. Using these images, the permeability rate was calculated using the OCT signal slope (OCTSS) method (explained in [16]). Briefly, after selection of a region inside tissue sample from the 1D OCT signals (z_{region}) and calculating the time interval for molecular diffusion inside this region (t_{region}) (the diffusion of dye induced time-dependent changes in tissues' optical properties), the permeability rate (\bar{P}) was found by dividing the thickness of the selected region by the time it took for the dye to diffuse ($\bar{P} = z_{\text{region}}/t_{\text{region}}$).

The histological examination of the time-dependent dye diffusion was done using a standard fluorescent microscope (Olympus BX61). A series of excised 1 cm² square aorta samples were taken from saline solution and placed on separate glass slides where 0.5 ml of dye solution was added simultaneously. Zero concentration of the dye was applied to one control sample. The control and tissues with the dye were situated on a 35 mm diameter cryocassette disc (Thermo Electron Corpora-

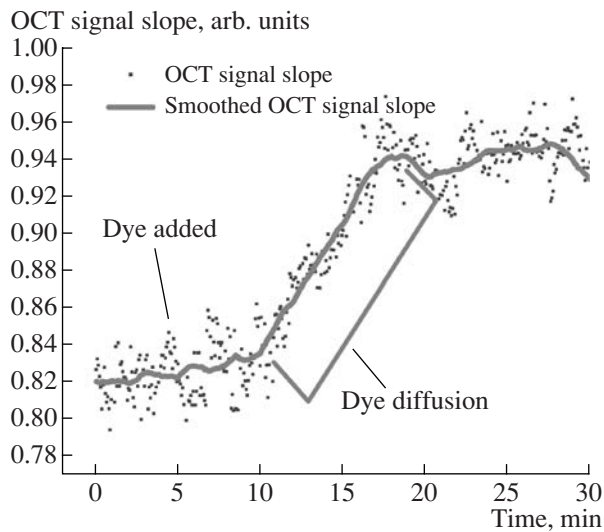


Fig. 2. OCT signal slope graph of dye diffusion experiment in pig aorta.

tion, Pittsburg, PA) and covered with cryochrome (Thermo Electron Corporation, Pittsburg, PA). The disc was then placed on dry ice where the cryochrome froze. Once frozen, the aorta tissues were then segmented laterally in $10\ \mu\text{m}$ slices that were mounted on microscope slides for imaging. These tissue samples were frozen at different time increments after the addition of the dye solution (10, 15, 20, 25, and 30 min). The same procedure was repeated to prepare slides for all tissue samples.

All the images from the fluorescence microscope were normalized to the maximum value from the control sample, thus eliminating the background autofluorescence created by the tissue itself. The distance that

the dye travelled in the tissue was measured at each time increment, and the permeability rate of the fluorescent dye was estimated by dividing the distance travelled at each time increment.

Also, all experimental procedures with both OCT and microscopic imaging were followed identically. Several trials were done concurrently with tissues from the coronary artery to ensure least variances that could be introduced from using samples from different places. All experiments were performed with minimized lighting conditions to avoid photobleaching of the dye.

RESULTS AND DISCUSSION

Figure 2 represents a sample OCTSS graph for a pig aorta during a dye diffusion experiment. The tissue was first imaged for about 5 min before adding 0.5 ml of the dye solution. A region inside artery of $140\ \mu\text{m}$ in thickness (z_{region}) was chosen to calculate the permeability. From Fig. 2, it is evident that the absorption coefficient increased as the dye solution reached the monitored region, as reflected in a rise in the OCTSS. After the dye has penetrated through the entire monitored region in the tissue, a saturation stage was reached which is illustrated by a flat line in the OCTSS graph. Taking the region where the slope increases in the OCTSS graph provides the time interval for the dye to diffuse through the indicated area, which is estimated to be approximately 9 min. From this information, the permeability rate was computed to be $2.59 \times 10^{-5}\ \text{cm/s}$.

Figures 3a–3f present normalized images of dye diffusion in pig aorta at different times using the fluorescent microscope method. A control image where no dye was introduced is shown in Fig. 3a. Figures 3b–3f are the images of dye in aorta at 10, 15, 20, 25, and 30 min,

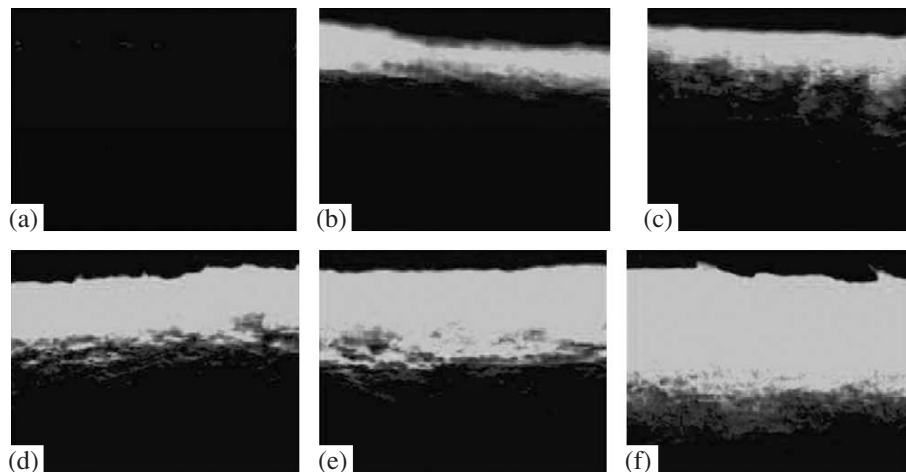


Fig. 3. Microscope images of pig aorta in a dye diffusion experiment at different times (a–f) images at 0, 10, 15, 20, 25, and 30 min.

respectively. The distances that the dye travelled in these images were measured to be 120, 181, 191, 263, and 350 μm which corresponded to an average permeability rate of 1.86×10^{-5} cm/s.

The permeability rate of the dye was found to be $(2.45 \pm 0.46) \times 10^{-5}$ cm/s ($n = 3$) from the OCT method and $(2.08 \pm 0.31) \times 10^{-5}$ cm/s ($n = 8$) from the fluorescence microscope examination. The obtained results from OCT experiments illustrate good agreement to those acquired using the fluorescent microscope. However, the slight difference in the averaged values and standard deviations from the two experimental methods could be related to the mechanical changes the aorta tissues underwent in the fluorescent microscope trials. It is well-known that freezing and mechanical slicing of the samples could alter its thickness attributing to variations in estimation of the dye diffusion region. Additionally, the average refractive index (RI) for aorta tissues was assumed to be 1.4 in all calculations performed with OCT method. This assumption is not entirely correct for two main reasons. First, the refractive index inside the aorta is nonuniform having slightly different values of RI for different tissue layers. Second, the diffusion of the dye into the monitored region will dynamically change the local value for the RI. However, we estimated that the change in the optical thickness of the selected region entitled from the change in the RI is small enough and was neglected in calculations of the permeability rate. However, a correction model for the RI in-depth distribution in layered tissues and its dynamics during molecular diffusion processes is being developed and will be engaged in our future studies.

Overall, the results obtained from both the OCT and fluorescent microscope suggest that OCT is a valid choice as a technique for measuring the molecular diffusion through biological tissues completely non-destructively and could be a good candidate for clinical application in the future.

CONCLUSIONS

In this paper we demonstrated that the quantification of molecular diffusion in tissues made with OCT completely non-destructively are comparable to already widely accepted techniques. Diffusion monitoring of a fluorescent dye was measured with OCT as well as traditional fluorescence microscope and the results were found to be in good agreement. This validates the notion for the use of the OCT-based noninvasive and non-destructive accurate assessment of the permeability rate of analyte in biological tissues.

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