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


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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 agent, 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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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 diffuse 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 utilizing 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 (P̄) was found by dividing the thickness of the selected region by the time it took for the dye to diffuse (P̄ = z_region/t_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-