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Terahertz spectroscopy of human sclera

Joong Wook Lee [a], Yong-Soo Lee [b], Jung-Min Park [a], Dae-Cheol Shin [a], Gyeong Bok Jung [c], Jae-Ho Shin [d], Soan Kim [b], Chul-Sik Kee [b, e], Chul Kang [b, *]

[a] Department of Physics and Optoelectronics Convergence Research Center, Chonnam National University, Gwangju 500-757, Republic of Korea
[b] Advanced Photonics Research Institute, GIST, Gwangju 500-712, Republic of Korea
[c] Department of Biomedical Engineering & Healthcare Industry Research Institute, College of Medicine, Kyung Hee University, Seoul 130-701, Republic of Korea
[d] Department of Ophthalmology, College of Medicine, Kyung Hee University, Seoul 130-701, Republic of Korea
[e] Center for Subwavelength Optics, Seoul 151-747, Republic of Korea

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ABSTRACT

Structural properties of human scleral tissues are investigated using polarization-dependent terahertz time-domain spectroscopy. Cross-linked tissue shows polarization-independent transmission properties, whereas normal tissue is polarization-dependent. This results from the different structural arrangements of the collagen fibrils that compose the human scleral tissues. In cross-linked tissue, collagen fibrils structurally form interlocking arrangements, unlike the regular parallel arrangement found in normal tissue. Our results demonstrate that terahertz spectroscopy is a powerful tool for investigating the structural characteristics of biological tissues and systems.

Understanding the optical properties of biological tissues is crucial for several biomedical optics and laser diagnostics applications, particularly for the study of the human scleral tissue, commonly known as the white of the eye. Most of the human eyeball is covered with scleral tissue that forms the opaque, protective, outer shell. This tissue is mainly composed of collagen fibers in the form of compact protein bundles. These collagen fibers are predominantly parallel [1,2]. Their diameters range from several dozen nanometers to several hundred nanometers and the fibers are densely packed so that their center-to-center distance is on the order of 200 nm [3].

The structural and mechanical characteristics of collagen fibers play a role in ocular conditions such as myopia. Highly myopic human eyes exhibit a scleral thinning of collagen fibers, and myopia is associated with changes in the diameter of scleral collagen fibrils [4]. Several researchers have attempted to slow down the progression of high myopia in various clinical trials involving scleral reinforcement operations as well as by attempting to increase the biomechanical rigidity efficiency of scleral collagens. This rigidity originates from cross-linked scleral tissue [4–8]. Despite the availability of such methods, little is known still about the structural characteristics of scleral collagens. Recently, G. B. Jung et al. examined the different structural properties of normal and cross-linked human scleral tissues by Raman spectroscopy, atomic force microscopy, and histology [9,10]. Their study revealed that cross-linked scleral tissue is arranged in interlocking shapes whereas normal tissues show a regular parallel arrangement.

In this study, we used terahertz (THz) time-domain spectroscopy (TDS), a useful technique for studying biological tissues and determining optical parameters such as their complex refractive index [11–16], to support further the finding that normal scleral tissue and cross-linked scleral tissue, formed of collagen fibrils, are arranged, respectively, along one direction and in interlocking shapes. The transmission of THz waves through the normal tissue aligned along one direction depends on the polarization direction, whereas in the case of cross-linked scleral tissue, it is polarization-independent because the interlocking shapes exhibit an approximate four-fold rotational symmetry [17]. Therefore, THz TDS can distinguish between normal and cross-linked scleral tissues while enabling the investigation of their structural characteristics.

Sclera samples were obtained from the Eye Bank of Kyung Hee University Medical Center, Seoul, Korea. Cross-linked scleral tissue samples were prepared by treatment with the photosensitizer riboflavin and illuminated with ultraviolet A (UVA, of relatively long wavelength). First, 0.1% riboflavin photosensitizer solution was injected into normal scleral tissue for 10 min. UVA light with a

* Corresponding author.
E-mail address: iron74@gist.ac.kr (C. Kang).

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radiant flux density of 3 mW/cm^2 and wavelength of 370 nm irradiated the samples for 30 min. Details of the sample preparation are provided in Ref. [9].

We used a standard THz TDS with a spectral range of 0.05–2 THz that was based on a femtosecond Ti:sapphire laser [18,19]. In this system, THz waves were generated by a photoconductive antenna and detected via electro-optic sampling. The sample surface was normal to the propagation direction of the waves. The polarization dependence of the transmitted waves was measured by rotating the samples. Frequency-domain spectra were obtained by a Fourier transform of the measured time traces. These spectra were then normalized over the entire frequency range by the spectrum of the THz signal transmitted through bare cover glass.

Fig. 1 shows topographical and three-dimensional images obtained with an atomic force microscope (AFM) (N9524A, Agilent Technologies) operated in non-contact mode at room temperature. Fig. 1(a) and (b) show normal scleral tissue, while Fig. 1(c) and (d) show cross-linked scleral tissue. In Fig. 1(a) and (b), we observe that the normal scleral tissue contains parallel collagen fibrils with nodular-like patterns visible in each fibril. The spatial arrangement of the collagen fibrils in normal scleral tissue is fairly regular and periodic; they appear to form a nano-grating-like structure. On the contrary, the collagen fibrils in the cross-linking tissue are not uniformly aligned in one direction; instead, they are primarily aligned along the directions labeled A and B in Fig. 1(d). It has been speculated that the multi-directional arrangement of collagen fibrils is associated with the formation of cross-links between the collagen molecules within the fibrils [20,21].

The time-domain waveforms of the THz waves were obtained by THz TDS. The insets of Fig. 2(a) and (b) show the time-domain waveforms of the THz wave transmission through the samples. The reference signal was measured without the samples along exactly the same path as that in the case of signals transmitted through the scleral tissue samples. The time delay between the reference and the signals shown in the inset of Fig. 2(a) is approximately 0.28 ps, which corresponds to a sample thickness of 168 μm, assuming a refractive index of 1.5 for human scleral tissue [22]. Fig. 2 shows the amplitude spectra obtained by Fourier-transforming the time-domain waveforms in the spectral range of 0.1–1.5 THz. In the case of normal scleral tissue in Fig. 2(a), the polarization angle θ is set to zero when the line A in Fig. 1(b) and the polarization of the incident THz waves are mutually perpendicular. The THz waves that are polarized parallel to the direction of collagen fibrils are transmitted to a lesser degree through normal scleral tissue than when they are polarized perpendicular. In the case of the cross-linked scleral tissue in Fig. 2(b), the polarization angle θ is chosen randomly. The difference between the transmission spectra measured at angles of 0° and 90° through the cross-linked scleral tissue is significantly smaller than in the case of the normal tissue.

To clarify the degree of transmission change on the polarization of the THz waves, we defined the parameter R as the ratio between the difference of the transmission amplitude spectra measured at the polarization angles of 0° and 90°, $E_0(\nu)$and $E_{90}(\nu)$, and a reference amplitude spectrum measured in the absence of the samples, $E_{ref}(\nu)$:

$$R(\nu) = \frac{\left|E_0^2(\nu) - E_{90}^2(\nu)\right|}{E_{ref}^2(\nu)}.$$

Fig. 3 plots R for normal and cross-linked scleral tissues. Normal scleral tissue is strongly polarization-sensitive at all frequencies, but cross-linked tissue is not.

THz waves polarized perpendicular to the collagen fibrils are transmitted to a greater degree than those parallel. The polarization sensitivity of normal scleral tissue is similar to that of a metallic

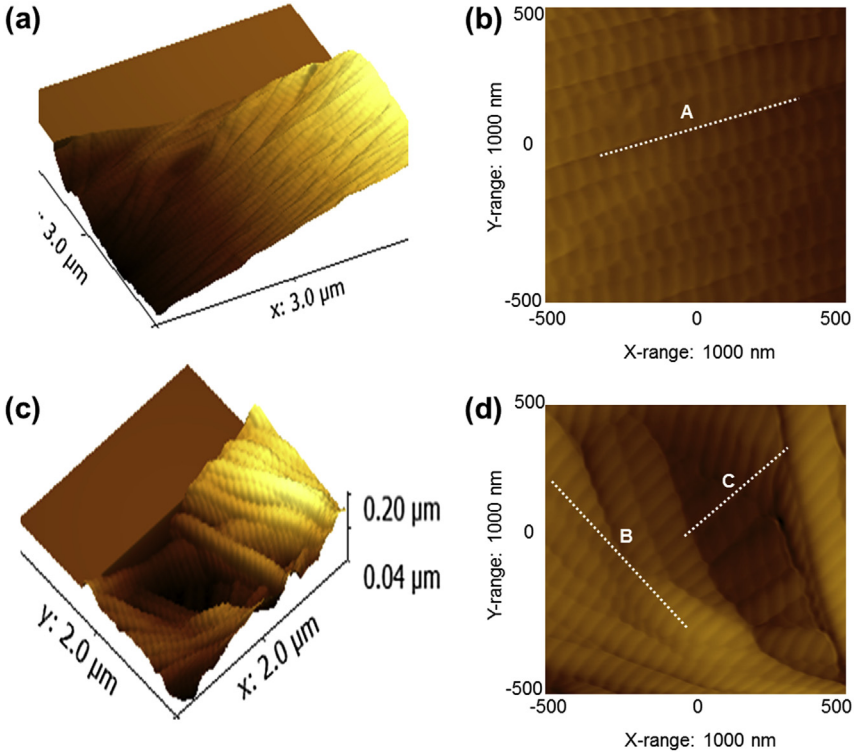

Fig. 1. (a) Three-dimensional AFM profile and (b) AFM topography of normal human scleral tissue. (c) and (d) are the corresponding results for cross-linked human scleral tissue. The lines A, B, and C represent the directions of the spatial arrangements of fibrils within human scleral tissue.