Quantitative analysis of water distribution in human articular cartilage using terahertz time-domain spectroscopy

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Abstract: The water distribution in human osteoarthritic articular cartilage has been quantitatively characterized using terahertz time-domain spectroscopy (THz TDS). We measured the refractive index and absorption coefficient of cartilage tissue in the THz frequency range. Based on our measurements, the estimated water content was observed to decrease with increasing depth cartilage tissue, showing good agreement with a previous report based on destructive biochemical methods.

OCIS codes: (110.6795) Terahertz imaging; (000.1430) Biology and medicine.

References and links

1. A. R. Poole, T. Kojima, T. Yasuda, F. Mwale, M. Kobayashi, and S. Laverty, "Composition and structure of articular cartilage: a template for tissue repair," Clin. Orthop. Relat. Res. **391**(391 Suppl), S26–S33 (2001).
2. Y. Xia, T. Farquhar, N. Burton-Wurster, E. Ray, and L. W. Jelinski, "Diffusion and relaxation mapping of cartilage-bone plugs and excised disks using microscopic magnetic resonance imaging," Magn. Reson. Med. **31**(3), 273–282 (1994).
3. C.-B. James and T. L. Uhl, "A review of articular cartilage pathology and the use of glucosamine sulfate," J. Athl. Train. **36**(4), 413–419 (2001).
4. H. J. Mankin and A. Z. Thrasher, "Water content and binding in normal and osteoarthritic human cartilage," J. Bone Joint Surg. Am. **57**(1), 76–80 (1975).
5. C. Liess, S. Lüsse, N. Karger, M. Heller, and C.-C. Glüer, "Detection of changes in cartilage water content using MRI T2-mapping in vivo," Osteoarthritis Cartilage **10**(12), 907–913 (2002).
6. S. Lüssea, H. Claassen, T. Gehrke, J. Hassenpflug, M. Schünke, M. Heller, and C.-C. Glüer, "Evaluation of water content by spatially resolved transverse relaxation times of human articular cartilage," Magn. Reson. Imaging **18**(4), 423–430 (2000).
7. B. B. Hu and M. C. Nuss, "Imaging with terahertz waves," Opt. Lett. **20**(16), 1716–1718 (1995).
8. B. Ferguson and X.-C. Zhang, "Materials for terahertz science and technology," Nat. Mater. **1**(1), 26–33 (2002).
9. M. Tonouchi, "Cutting-edge terahertz technology," Nat. Photonics **1**(2), 97–105 (2007).
10. P. H. Siegel, "Terahertz technology in biology and medicine," IEEE Trans. Microw. Theory Tech. **52**(10), 2438–2447 (2004).
11. D. Crawley, C. Longbottom, V. P. Wallace, B. Cole, D. Arnone, and M. Pepper, "Three-dimensional terahertz pulse imaging of dental tissue," J. Biomed. Opt. **8**(2), 303–307 (2003).
12. R. M. Woodward, V. P. Wallace, R. J. Pye, B. E. Cole, D. D. Arnone, E. H. Linfield, and M. Pepper, "Terahertz pulse imaging of *ex vivo* basal cell carcinoma," J. Invest. Dermatol. **120**(1), 72–78 (2003).
13. V. P. Wallace, A. J. Fitzgerald, S. Shankar, N. Flanagan, R. Pye, J. Cluff, and D. D. Arnone, "Terahertz pulsed imaging of basal cell carcinoma *ex vivo* and *in vivo*," Br. J. Dermatol. **151**(2), 424–432 (2004).
14. M. R. Stringer, D. N. Lund, A. P. Foulds, A. Uddin, E. Berry, R. E. Miles, and A. G. Davies, "The analysis of human cortical bone by terahertz time-domain spectroscopy," Phys. Med. Biol. **50**(14), 3211–3219 (2005).
15. A. J. Fitzgerald, V. P. Wallace, M. Jimenez-Linan, L. Bobrow, R. J. Pye, A. D. Purushotham, and D. D. Arnone, "Terahertz pulsed imaging of human breast tumors," Radiology **239**(2), 533–540 (2006).
16. E.-A. Jung, M.-H. Lim, K.-W. Moon, Y.-W. Do, S.-S. Lee, H.-W. Han, H.-J. Choi, K.-S. Cho, and K.-R. Kim, "Terahertz pulse imaging of micro-metastatic lymph nodes in early-stage cervical cancer patients," J. Opt. Soc. Korea **15**(2), 155–160 (2011).

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17. W.-C. Kan, W.-S. Lee, W.-H. Cheung, V. P. Wallace, and E. Pickwell-Macpherson, "Terahertz pulsed imaging of knee cartilage," Biomed. Opt. Express **1**(3), 967–974 (2010).
18. Rønne, L. Thrane, P.-O. Åstrand, A. Wallqvist, K. V. Mikkelsen, and S. R. Keiding, "Investigation of the temperature dependence of dielectric relaxation in liquid water by THz reflection spectroscopy and molecular dynamics simulation," J. Chem. Phys. **107**(14), 5319–5331 (1997).
19. S. L. Chuang, *Physics of Optoelectronic Devices* (Wiley Interscience, New York, 1995), p. 209.
20. R. Brocklehurst, M. T. Bayliss, A. Maroudas, H. L. Coysh, M. A. Freeman, P. A. Revell, and S. Y. Ali, "The composition of normal and osteoarthritic articular cartilage from human knee joints. With special reference to unicompartmental replacement and osteotomy of the knee," J. Bone Joint Surg. Am. **66**(1), 95–106 (1984).
21. E. M. Shapiro, A. Borthakur, J. H. Kaufman, J. S. Leigh, and R. Reddy, "Water distribution patterns inside bovine articular cartilage as visualized by ^{1}H magnetic resonance imaging," Osteoarthritis Cartilage **9**(6), 533–538 (2001).

1. Introduction

Osteoarthritis (OA), one of the most prevalent chronic diseases in the elderly, is characterized by progressive degeneration of cartilage. Cartilage degeneration is affected by biochemical alterations, including an increase in water content and the loss of proteoglycans [1–3]. Several studies have shown that the water content in osteoarthritic cartilage may increase by about 10% [4]. Therefore, a precise measurement of the water content in cartilage can aid in the diagnosis of early-stage OA. However, changes in the water content in the early stages of OA cannot be detected using current clinical techniques such as radiography and arthroscopy. Only magnetic resonance imaging (MRI) has been used for the detection of water content in the early stages of OA [5,6].

Terahertz time-domain spectroscopy (THz TDS) has recently been developed because of recent advances in THz technology. THz TDS is a coherent and non-ionizing method that can quantify the complex refractive index from both the phase and amplitude information of a medium [7–9]. Moreover, this method can also probe low frequency vibrational modes of biomolecules, thus providing structural and functional information about biological tissue [10]. Because water has strong absorptions across the entire THz frequency range, THz images will likely show a good image contrast dependent on the changes in medium water content. This enables THz TDS to be used for spectroscopic investigation of a biological medium.

To date, several biological tissues have been examined using this technique. For instance, characterization of human dental tissues [11], basal cell carcinoma from both *ex vivo* and *in vivo* samples [12,13], and human cortical bone [14] has been reported. More recently, human breast tumors [15] and micro-metastic lymph nodes [16] have been successfully investigated using THz TDS although the clinical application of THz TDS has not been demonstrated due to the high water absorption. However, no literature is available on the quantitative analysis of human articular cartilage in the THz region. Only THz reflection images of rabbit cartilage have been reported [17]. Here we report on the THz characterization of water distribution in human articular cartilage.

2. Materials and methods

Human osteoarthritic articular cartilage tissues were obtained from the Department of Orthopedic Surgery at Ajou University Hospital, Korea. The tissue diagnosed as OA was excised from a patient after total knee joint replacement. Appropriate consent was obtained for the measurements and all materials were returned to the Ajou University Hospital for disposal after the measurements. The articular surface of the cartilage tissue was visually intact. Using a razor blade, the excised cartilage tissue was cut into a slice (622 ± 30 µm) to study the depth information from the articular surface to the subchondral bone, as depicted in Fig. 1(a), where the thickness was measured by a digital thickness gauge with an accuracy of 1 µm. The sliced cartilage was placed on a 150-µm-thick glass slide and covered with a 10-µm-thick film of low density polyethylene (LDPE) to prevent desiccation (Fig. 1(b)).

The experimental setup was based on a conventional TDS system with transmission geometry. The THz pulse was generated by an InAs wafer pumped by a Ti:sapphire laser with a center wavelength of 790 nm, a pulse width of 100 fs, and a repetition rate of 80 MHz. The

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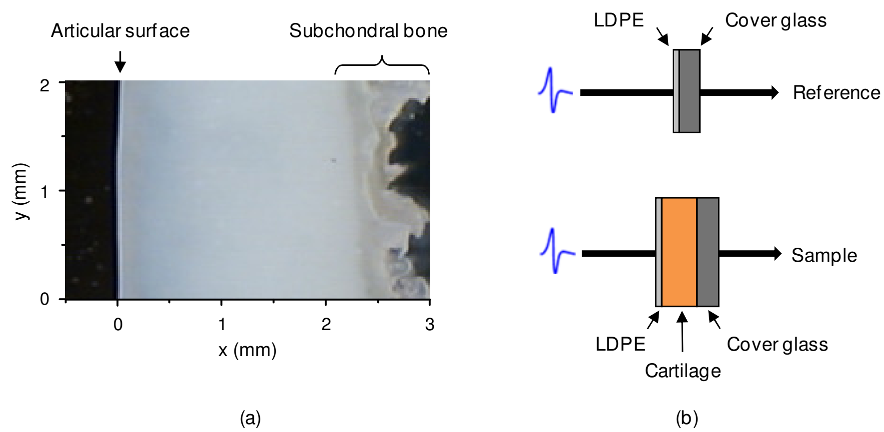

Fig. 1. (a) Optical image of human articular cartilage tissue. (b) Schematic of reference and cartilage samples.

generated THz pulse was collimated and focused by off-axis parabolic mirrors. The cartilage sample was placed at the THz beam waist and moved on a motorized stage between two off-axis parabolic mirrors. The focal length of a set of off-axis parabolic mirrors was 5 cm. The scanned area was 3.5 × 2 mm^2, and the scanning steps of the horizontal (x) and vertical (y) directions were 0.3 and 1 mm, respectively. The transmitted THz signal was detected by a photoconductive antenna fabricated on a low-temperature grown GaAs using standard optical gating and phase-sensitive detection techniques.

3. Results and discussion

Figure 2 shows the THz pulse signals and amplitude spectra with and without cartilage tissue with the transmitted THz pulses recorded at the center of cartilage sample (x = 1.0 and y = 1.0 mm). The transmitted THz pulse for the cartilage sample was significantly attenuated by absorption and Fresnel loss, and was ~10 times smaller than that of the reference signal. As a THz pulse propagates through an absorptive medium, such as a biological medium, the pulse width broadens due to the dispersion. The spectral amplitude transmitted through the cartilage tissue was found to be reduced over the entire THz frequency range (Fig. 2(b)).

Figure 3 shows the frequency-dependent refractive indices and absorption coefficients of cartilage tissue along its depth. The dotted lines indicate the refractive index and absorption coefficient of pure water, as reported in Ref. [18]. The refractive index and absorption coefficients near the articular surface were not included in Fig. 3 because of the diffraction effect at the edge of the sample. Over the entire frequency range, the refractive indices and

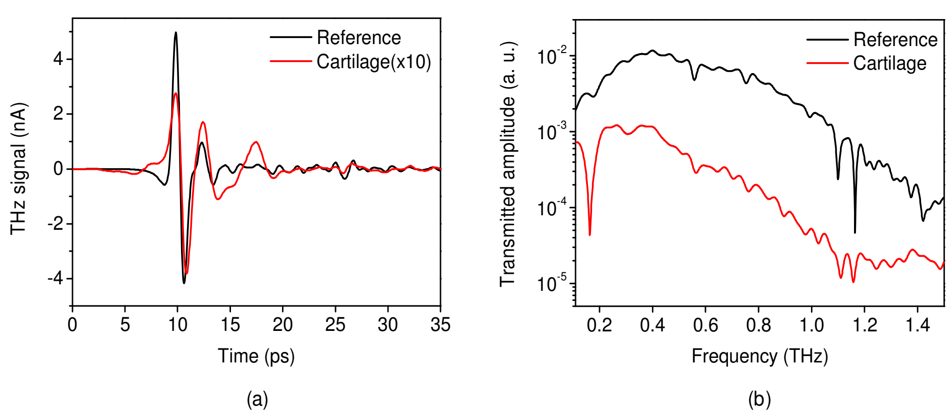

Fig. 2. THz signals and transmitted amplitudes of reference and cartilage tissue.

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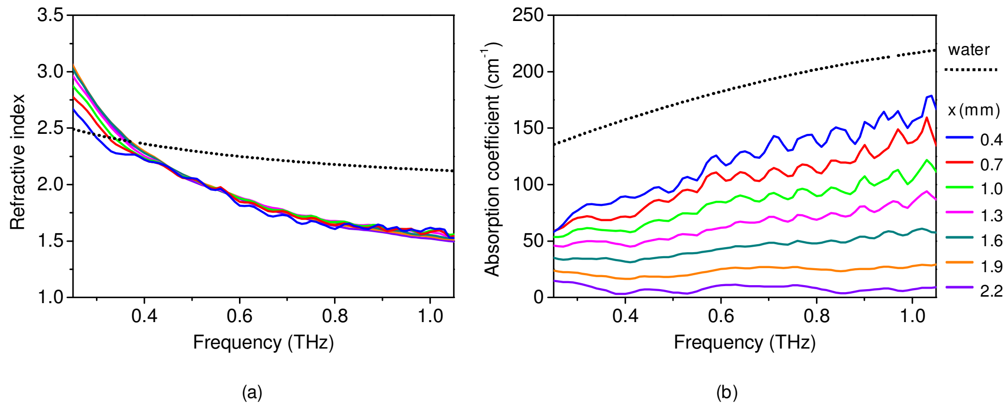

Fig. 3. Frequency dependence of (a) the refractive index and (b) the power absorption coefficient along the depth of the cartilage tissue. Each dotted line indicates the values for liquid water as reported in a previous literature [18].

absorption coefficients of the cartilage tissue gradually decreased and increased, respectively. Each absorption coefficient along the depth was lower than that of liquid water. In addition, no significant change in the refractive indices along the depth of the cartilage was observed. However, we found that the absorption coefficients decreased from the articular surface to the subchondral bone. For the extraction of the complex refractive index, we used an iteration method based on the transfer matrix theory where the effects of multiple reflections at the interfaces between the slide, cartilage, and LDPE film are taken into account [19].

Figure 4 shows the refractive index images and absorption coefficient images of cartilage tissue at 0.4 and 0.8 THz. The refractive index was relatively constant along the depth of cartilage at both 0.4 and 0.8 THz with the exception of the surface of the cartilage because of the diffraction. In the absorption coefficient image of the cartilage, the absorption was high at

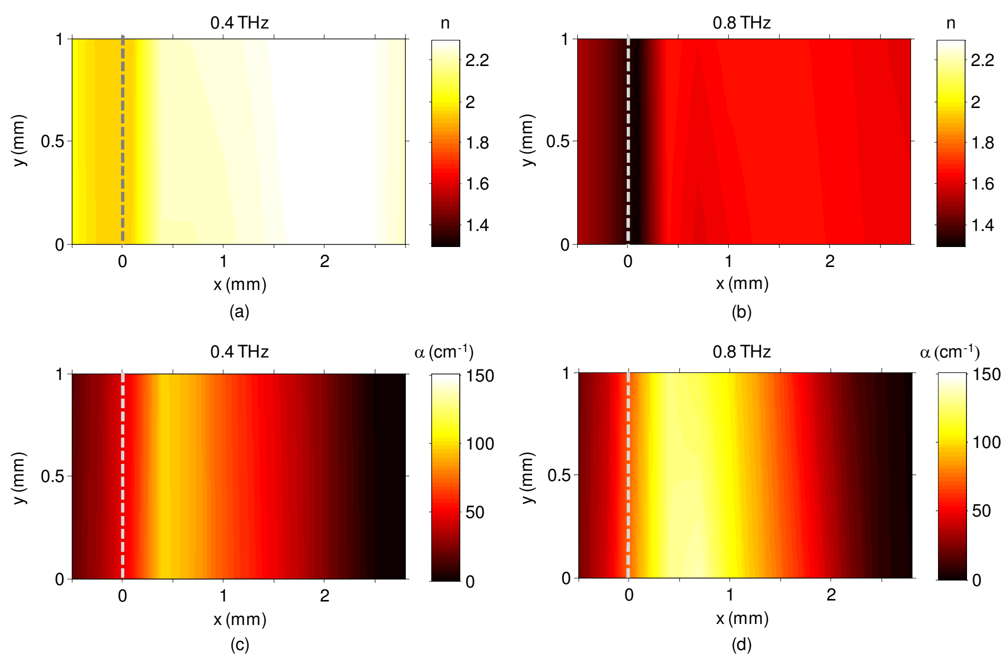

Fig. 4. Refractive index images and absorption coefficient images of articular cartilage at 0.4 and 0.8 THz. The dashed lines indicate the cartilage surface.

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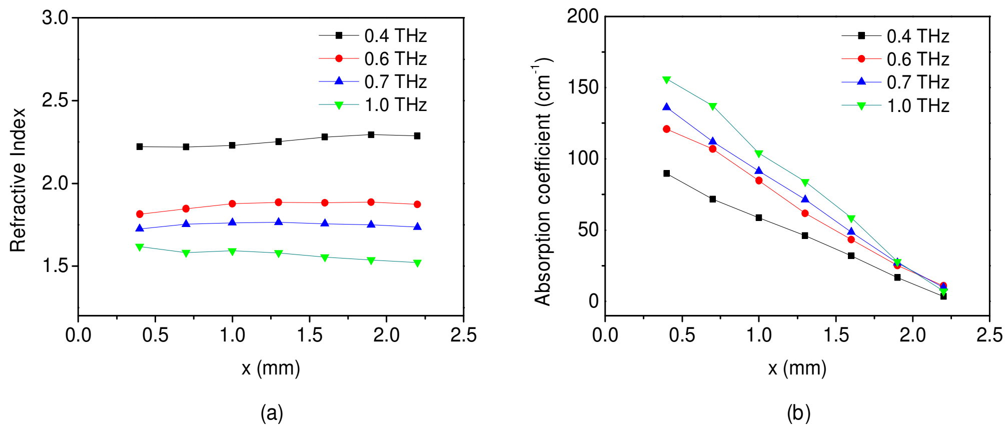

Fig. 5. (a) Refractive index profile and (b) absorption coefficient profile along the depth of cartilage tissue at 0.4 and 0.8 THz.

the articular surface and gradually decreased along the depth of the cartilage. The refractive indices and absorption coefficients along the depth of cartilage at specific frequencies are shown in Fig. 5. At each frequency, the difference between the maximum and minimum values of the refractive index was less than 5% along the depth. In contrast, the absorption coefficient at each frequency significantly decreased from the articular surface to the subchondral bone. It has been known that the cartilage tissue is spatially heterogeneous and molecular composition of cartilage varies significantly in going from the articular surface to subchondral bone [1–6]. Therefore we speculate that the alteration of absorption coefficient along the depth of the cartilage matrix may result primarily from changes in water content because water has a strong absorption in the THz frequency range.

The effective absorption coefficient of cartilage tissue is related to the absorption coefficients of the components in the cartilage, including water, proteoglycans, and collagen. To characterize the water distribution in cartilage tissue from the absorption coefficient, we should in principle take into account all the effects of the biochemical components in cartilage. However, we assumed that the absorption coefficient was determined predominantly by the water content, and did not account for other components, since water has a much higher THz absorption than the other biochemical components in cartilage. Consequently, we also assumed that the absorption coefficient was almost proportional to the volume fraction of

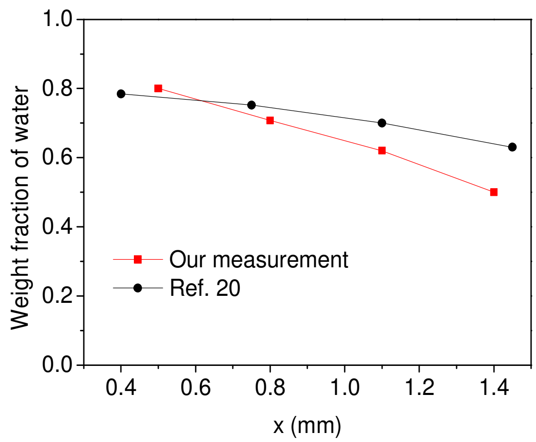

Fig. 6. Weight-fractional distribution of water in cartilage. The red and black curves represent the measurements by THz TDS and destructive biochemical method [20], respectively. The first points in the two curves correspond to the cartilage surfaces.

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the water. Since the articular cartilage sample was diagnosed as osteoarthritic tissue but still had a visually intact surface, we compared our measurements with the water content of cartilage with an intact surface reported in a previous study [20] that used a destructive biochemical method to measure the water content. The calculated volume fraction of water was converted to a weight fraction using the conversion relation described in Ref. [21]. As seen in Fig. 6, the water content in our measurement decreased along the depth and shows a reasonably good agreement with the values from this previous study [20]. Further, the estimation of the water distribution in bovine cartilage using MRI demonstrated that water content varies from ~86% on the articular surface to ~63% on the subchondral bone [21], showing reasonably good agreement with our measurements.

4. Conclusion

We measured the refractive index and absorption coefficient of human articular cartilage, and quantitatively characterized the water distribution in cartilage matrix using THz TDS. The absorption coefficient of the cartilage tissue gradually decreased along the depth in the THz frequency range. The water content in our measurement shows reasonably good agreement with that of a previous report based on a destructive biochemical method. This suggests that the molecular composition, or more specifically, the water content, in cartilage matrix might have a specific depth profile that is correlated with the degree of degeneration in cartilage, which can possibly be measured by THz TDS.

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