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Ultrasonic and Photoacoustic Imaging of Knee Joints in Normal and Osteoarthritis Rats

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***Abstract*— Osteoarthritis (OA) is a common joint disorder and estimated to cause symptoms in 20-40% of the elderly population. 532 nm laser is much absorbed in developed vascular network in the spongy bone which is one of the main characteristics of OA. In this study, a photoacoustic (PA) imaging system with 532 nm laser and 50 MHz US (ultrasound) transducer was developed. Normal and OA knee joints were observed by US and PA imaging. PA signal from the spongy bone was strong where US reflection was very weak. PA signal from the spongy bone was significantly strong in OA compared with that in normal knee while US showed similar low intensity echo in normal and OA. Detailed structure and information on vascular density of spongy bone in rat knee joint were successfully obtained with US / PA combined imaging. US / PA imaging should be useful for early diagnosis of OA.**

I. Introduction

The number of patients with osteoarthritis (OA) is about 24 million [1]. OA is a common joint disorder and estimated to cause symptoms in 20-40% of the elderly population in aging societies [2]. Main symptom of OA is pain, joint stiffness and muscle weakness [3]. Early detection and diagnosis are important for successful treatment of the disease [4]. Diagnostic modalities such as plain X-rays, computed tomography (CT), magnetic resonance imaging (MRI) and ultrasound (US) for OA are clinically applied. Demerit of plain X-rays and CT is the exposure of radiation. MRI is a powerful diagnostic tool for evaluating the knee cartilage, however, MRI is expensive and the number is limited for routine use [5]. US is quick, inexpensive and highest resolution imaging [6].

Photoacoustic (PA) method which is based on PA phenomenon [7-14] may realize high resolution and deep penetration imaging of the biological tissues. Beside US information on morphology, PA imaging provides information on vasculature which is one of main characteristics of the OA cartilage [15]. When a nano-second pulsed laser is illuminated to the biological tissue, light energy is absorbed. Local temperature rise leads to a thermal-elastic expansion and the generation of a pressure wave which is called as PA signal. The PA signal is then detected by ultrasonic transducer or hydrophone. Thus, PA signal depends on absorption coefficient of the tissues and the wavelength of the laser [12]. For example, strong signals are generated from blood vessels when the laser with the wavelength of 532 nm is used. Recently, venules and arterioles are differentiated by the difference of absorbency between HbO_2 and Hb_R [13] and those vessel networks are colorized by using various wavelength of the laser [14]. The amplitude of the PA signal is expressed by the absorption coefficient and the tissue elasticity. Therefore, PA imaging provides not only the morphology or structure but also the information on the color and elasticity of the tissue beneath the opaque layers.

In the present study, we develop a PA imaging system using a semiconductor laser for visualization of normal and OA knee joints of rat to clarify the PA characteristics of the knee cartilage by comparing with US and find the early sign of OA for further development of diagnostic device.

II. Methods

A. Experimental Setup

Figure 1 shows the block diagram of the system. Laser pulses were generated by a semiconductor laser (L11038-02Y, Hamamatsu Photonics, Hamamatsu, Japan) with the repetition rate of 50 Hz.

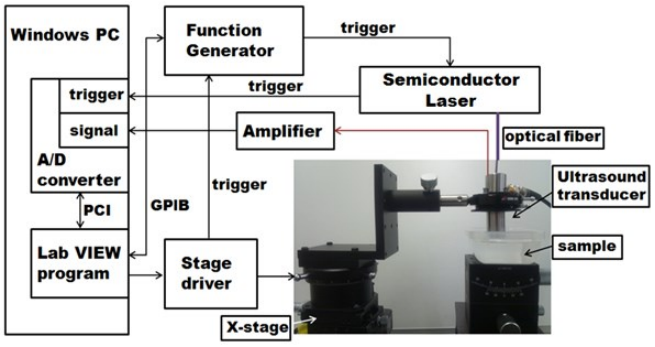

Figure 1. Block diagram of the photoacoustic imaging system

PA signal was received by a 50 MHz (-6dB bandwidth: 25-59 MHz) concave P(VDF-TrFE) ultrasound transducer shown in Figure 2. The aperture size was 4.5 mm, focus length was 9.0 mm, axial resolution was 30 μm and the lateral resolution was 61.2 μm. This had a hole in the central part to get through a light fiber in order to align light illumination and signal reception concentrically. Mechanical scanning of the

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transducer was realized by using an x-axis stage (SGSP20-20, Sigma-koki, Tokyo, Japan) and a stage-driver (Mark-202, Sigma-koki, Tokyo, Japan). A digitizer card (DP1400, Acqiris, Geneva, Switzerland) installed in the PC was used to acquire the PA signal with the sampling rate up to 1 GSample/s. Lab-VIEW program was used to control the stage-driver and the function generator (MF1944B, NF Corp., Yokohama, Japan) which sent a trigger to a semiconductor laser. Further data processing and analysis were conducted by using MATLAB program (MathWorks, Natick, MA, USA).

Figure 2. 50-MHz concave transducer with a hole for the optical fiber in the center

B. OA Model [16]

The protocol for the experiments was approved by the Animal Research Committee of the Tohoku University School of Medicine. Adult male Sprague-Dawley rats (body weight 380-400 g) were used in this study. The unilateral knee joint was immobilized by plastic plate and metal screws for 4 weeks. Figure 3 shows the X-ray photo of immobilized knee joint. Four rats were prepared for gross observation for articular cartilage. The immobilized animals and the sham operated animals made up the immobilized group and the control group, respectively.

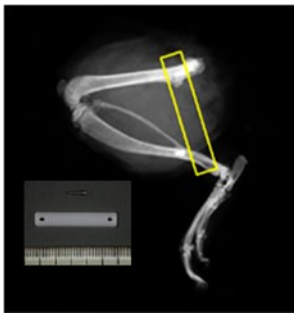

Figure 3. X-ray of immobilized knee joint

The knee capsule was cut with a surgical knife and the joint opened after administration of an overdose of sodium pentobarbital. After resection of ligaments and meniscus, a cartilage-bone complex (3.8 mm diameter) was obtained from the medial midcondylar regions of the tibia with a cylindrical bar (Trephine Bur, cat. No.13006, Technika, Tokyo, Japan).

III. RESULTS

Figure 4 shows the US and PA signals and the power spectra from the polyvinyl chloride phantom. US signal was detected about 20 μsec after the electric pulse was input to the US transducer. PA signal was received about 10 μsec after the trigger pulse from the laser.

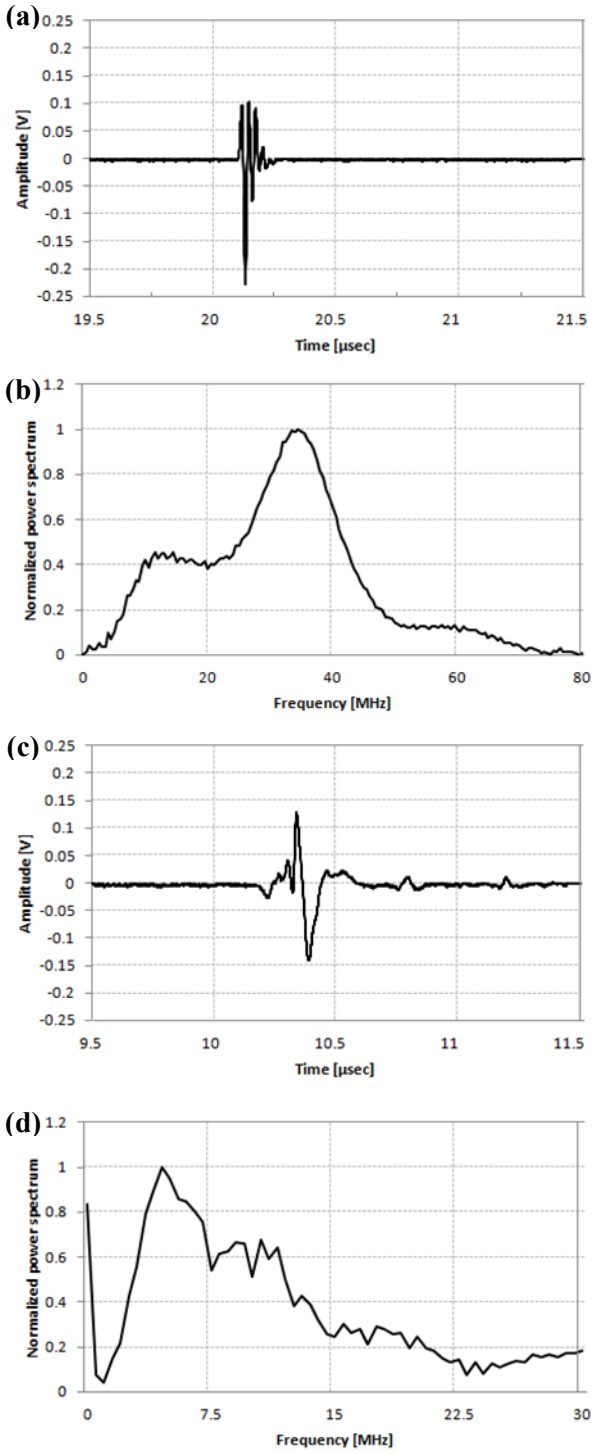

Figure 4. US / PA signals and power spectra
(a) US signal, (b) US spectrum, (c) PA signal, (d) PA spectrum

The difference was explained by the propagation speed of light and ultrasound. The speed of light in the water is 2.249×10^8 m/s and the speed of sound in the water is 1.540×10^3 m/s. The power spectrum showed steep peak at 35 MHz in US and wide spectrum between 3 and 14 MHz in PA.

Figure 5 shows the macroscopic view of the rat knee joint. The black square shows 2 x 2 mm and the region was observed from the top by PA / US imaging.

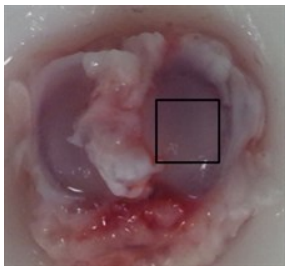

Figure 5. Macroscopic view of normal rat knee joint from the top

Figure 6 (a) shows a three-dimensional (3D) PA image merged with US image of a normal rat knee joint. An imaging plane of 2 x 2 mm (black square of Figure 5) was obtained at a scanning step of 40 μm. PA signals were averaged at 4 times to reduce a noise.

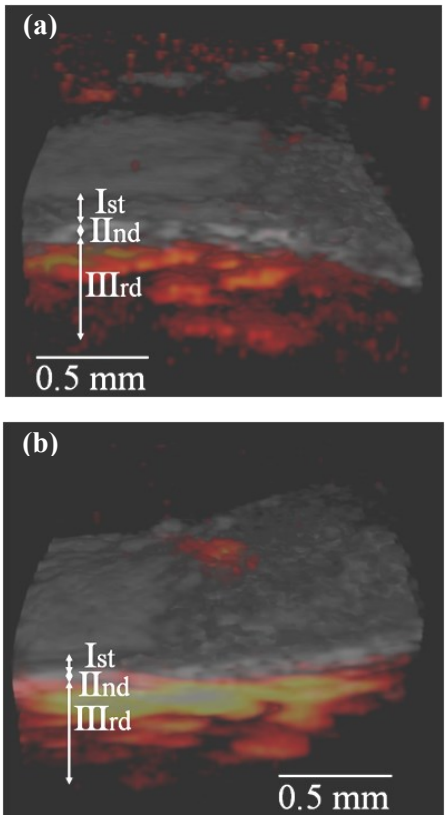

Figure 6. US / PA merged image of rat knee joint
(a) Normal, (b) OA model

In Figure 6 (a), Ist layer is cartilage, IInd layer is subchondral bone and IIIrd layer is a spongy bone in US imaging. US imaging is shown by gray scale and PA imaging is shown by color scale. The signal from the cartilage surface was weak and the signal from the surface of subchondral bone was strong in US imaging. On the other hand, signal from the cartilage was weak and the signal from the spongy bone was greater than that of subchondral bone in PA imaging. Figure 6 (b) shows a 3D US / PA merged image of an OA model. The US signal from the cartilage surface was weaker than that of normal knee and the PA signal from the spongy bone was greater than that of normal knee.

Figure 7 shows the average values of PA and US signal intensities. PA signal intensity from OA was significantly stronger than that from normal ($P < 0.05$). On the other hand, significant difference of US signal was not seen between OA and normal. Mann-Whitney's U test was used for the statistical analysis.

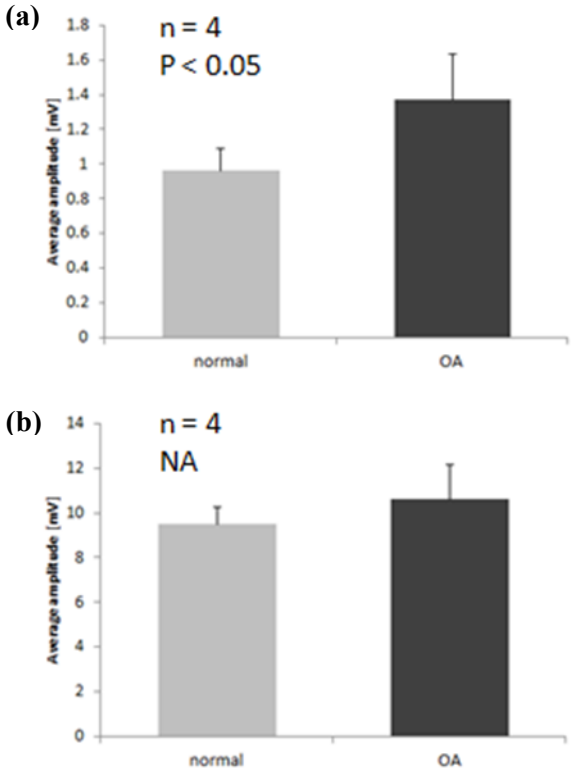

Figure 7. Bar graphs showing average intensities of PA and US in normal and OA rat knee joint
(a) PA, (b) US

IV. Discussion

In the present study, US / PA transducer which combined the optical fiber transmitting diode laser and the ultrasonic concave transducer with the central frequency of 50 MHz was used. The system successfully visualized the structure of knee joint consisted of cartilage, subchondral bone and spongy bone. The results indicate that the US reflection was strong at the surfaces of cartilage and subchondral bone because the difference of the specific acoustic impedance between neighboring layers was strong at those interfaces. On the other hand, US signal from the spongy bone was weak. US was almost reflected at the surface of the subchondral bone and it didn't penetrate into spongy bone. However, PA signal from

the spongy bone was very strong. The laser light with the wavelength of 532 nm was not absorbed in the cartilage and subchondral bone because they were lucent or white. The spongy bone is highly vascular and frequently contains red bone marrow where hematopoiesis, the production of blood cells, occurs. Laser light with the wavelength of 532 nm was much absorbed in the red color area to generate a strong US signal.

The other point is the frequency of the US and PA signals. The central frequency of the transmitted US was 50 MHz and the peak frequency of the received US signal was 35 MHz. On the other hand, the peak frequency of the PA signal generated by 3.2 ns pulsed laser was 5 MHz. Thus, PA signal was not attenuated in the bone and cartilage because the attenuation is proportional to the first to the second power of the frequency

In case of knee joint of rat OA model, PA signal in the spongy bone was significantly stronger than normal knee joint. In histological analysis, vascularization occurs in the spongy bone according to the OA progress [15]. Thus, PA signal from the spongy bone turned up with increased vascular density.

In the present study, the laser pulse energy was tuned to 420 μJ. Assuming that the depth of the laser focus was 2 mm, the calculated surface laser fluency was 16 mJ/cm^2. The energy was less than the 20 mJ/cm^2 which satisfied the safety standard set by American National Standards Institute (ANSI) [17].

For clinical application of PA for human knee joints, the wavelength of the laser should be tuned as 580-600 nm for deeper penetration. As well as the wavelength, US frequency should be 10-20 MHz because the frequency range of the PA signal was 3 and 14 MHz. The tradeoff of the frequency and the resolution will be discussed at the clinical phase.

V. Conclusion

A PA imaging system with 532 nm laser and 50 MHz US transducer was developed. The morphology of the cartilage-bone complex of rat knee joint was successfully obtained by US imaging. PA signal was strong in spongy bone in which US reflection was weak. The spongy bone of the OA model showed stronger PA signal than that in the normal knee joint. The combination of US / PA system provides not only microscopic morphology but also information on vascular distribution in the tissue. The size of the US / PA system would be much smaller than MRI or CT. Thus, the system may be applied for the screening and early diagnosis of the OA in the clinical settings.

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