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Ultrasonically induced electric potentials in extracted collagen from cortical bone
脱灰した皮質骨中の超音波誘発電位の検討

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1. Introduction

Clinical studies show that the ultrasound irradiation can reduce the time of bone fracture healing. The detailed mechanism of ultrasonic effects on bone, however, has not been clearly understood yet.

Concerning the bone healing, Fukada and Yasuda have reported that the mechanical stress at low frequencies induces electrical potentials in bone [1]. Possible mechanism of induced electrical potentials is considered as the streaming potential or the piezoelectricity. However, the streaming potential seems difficult to occur in the MHz range.

In order to evaluate the stress induced electrical potentials at high frequencies, we used cortical bone as a piezoelectric device [2, 3]. We have then fabricated a bone transducer, to obtain output signals induced by ultrasound irradiation in the MHz range. Here, type I collagen and hydroxyapatite (HAp) is the principal component of cortical bone. Type I collagen is an anisotropic tissue and is also known to have piezoelectricity.

In this study, we investigated the effect of the collagen in bone on the ultrasonically induced potentials. We also tried to evaluate the anisotropy in the collagen in the MHz range.

2. Material and Methods

2.1 Fabrication process

Cortical bone samples were extracted from the anterior part of the mid-femoral shafts of a 33 month-old bovine as shown **Fig. 1**. We fabricated three bone transducers with cortical bone samples. The diameters and thicknesses of these samples were 10.0 and 1.00 mm, respectively.

On the other hand, in order to obtain extracted collagen, we fabricated another three cortical bone samples. Then, decalcification to remove HAp from the bone was carried out using EDTA (Ethylene Diamine Tetra Acid) solution. In this process, samples were immersed for 7–10 days in EDTA solution at room temperature. The EDTA solution was not changed during the decalcification. After decalcification, we confirmed that there were no HAp crystallites in bone by the X-ray diffraction technique. The diameters of these samples were 10.0 mm and the thicknesses were A : 1.00, B : 0.80 and C : 0.65 mm, respectively. We fabricated three collagen transducers (A, B and C) with the decalcified bone samples.

2.2 Experimental methods

Figure 2 shows the experimental setup. A PVDF focus transducer (diameter, 20 mm; focal length, 40 mm; custom made by Toray) was used as a transmitter and a handmade transducer (PVDF, bone or collagen) was used as a receiver [4]. In the setup, a function generator (33250A; Agilent Technologies) generated an electric pulse, which was amplified to 70 $V_{p\text{-}p}$ by a bipolar power supply (HAS 4101; NF). The pulse was applied to the transmitter. Ultrasound was irradiated to the surface of bone or collagen sample. The received signal was amplified 40 dB by a pre-amplifier (BX-31A; NF) and observed in an oscilloscope (DPO3054; Tektronix). We performed

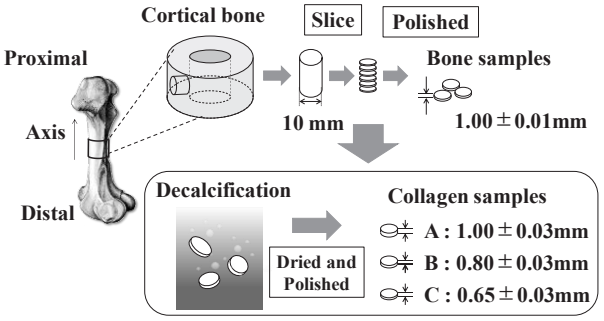
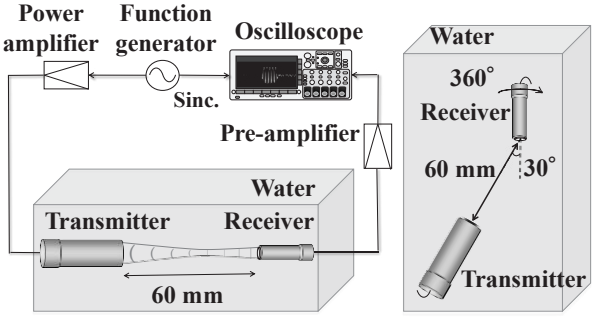


Fig. 1 Fabrication process of bone plate samples.



(a) (b)

Fig. 2 Experimental setup.

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two experiments. First, setting the transmitter and receiver coaxially, we irradiated 10 cycles of sinusoidal wave at 0.7-2.0 MHz to the receiver and observed induced potentials. The transmitted ultrasound pressure at the measurement point was around 10 kPa_{p-p}. Second, with some incident angles to the receiver (**Fig. 2(b)**), one cycle of square pulse wave at 10 kHz were applied to the transmitter and radiated short ultrasonic pulses. The anisotropic characters of induced potentials were observed by rotating the collagen transducer with each 10 degrees. The transmitted ultrasound pressure at the measurement point was around 1.3 kPa_{p-p}.

3. Results and Discussion

The transducers were perfectly dried before experiments. **Fig. 3** shows the observed wave forms obtained by the bone transducer and the collagen transducer near the resonance frequency. The sensitivities were around 1/1000 of the PVDF transducers. We measured the sensitivity of bone or collagen transducers **(Fig.4)**. The maximum sensitivity of the collagen transducer was about 8.54 nV/Pa and that of the bone transducer was about 1.50 nV/Pa. The maximum sensitivity of the collagen transducer was around 6 times larger than that of the bone transducer at the thickness resonance frequency. Induced electric potentials of collagen transducers were always larger than those of bone transducers. The results may indicate that the piezoelectricity of bone mainly comes from the collagen. The capacitance of the collagen transducer was almost 35% of the bone transducer. The results suggest that the actual piezoelectricity of collagen was much larger, because the small capacitance of the collagen transducer decreases the amplitudes of observed signals in the oscilloscope.

We evaluated the effect of collagen anisotropy on the induced electric potentials. **Fig. 5** shows the amplitude of the stress-induced electrical potentials as a function of rotating angle. The amplitudes became maximum around 10, 135, 250 and 315 degrees. The result is similar to the anisotropy of stress induced potentials in the cortical bone [5]. The collagen fibers play an important role in the piezoelectric anisotropy of bone.

4. Conclusion

We investigated induced electric potentials in the extracted collagen from cortical bone. The sensitivity of the collagen transducer was higher than that of bone transducer and showed anisotropy. The results may indicate that the piezoelectricity of bone mainly comes from the collagen.

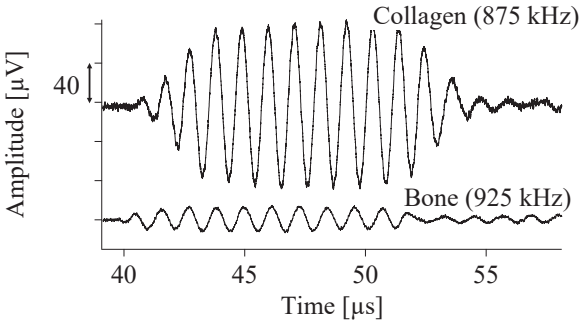

Fig. 3 Received waveforms at resonance frequency.

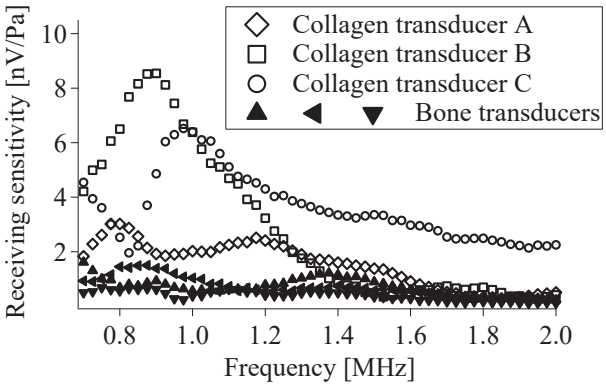

Fig. 4 Receiving sensitivity.

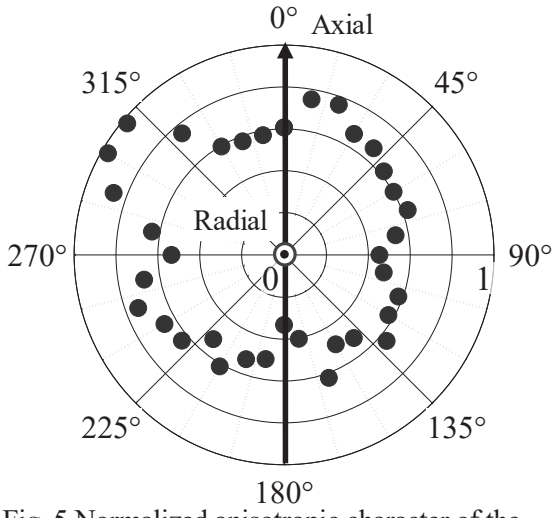

Fig. 5 Normalized anisotropic character of the induced electric potentials by the ultrasound irradiation.

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