Correction of Thickness Measurement by Ultrasound for Articular Cartilage Using Sound Velocity Estimation

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Abstract

Background. Ultrasound measurement of osteoarthritis cartilage thickness is not sufficiently accurate if constant sound velocity is used. The aim of this study was to investigate the applicability of estimating the sound velocity using echo reflectance from the cartilage surface. Methods. Change in measurement error of cartilage thickness was evaluated using collagenase-treated osteochondral plugs. Sound velocity was calculated from the reflectance ratio, and the reference thickness was measured using a needle method. Findings. The relative errors of thickness measurements using constant sound velocity were increased after collagenase treatment. The errors using calculated sound velocity showed no significant difference between intact and degenerated samples. Interpretation. The collagenase treatment-induced error was reduced from 7% to 2% in absolute value, suggesting the applicability of the correction method for ultrasound measurement.

Key words: Ultrasound, Cartilage, Thickness Measurement

1. Introduction

Ultrasound has been widely used in clinical practice for the noninvasive evaluation of soft tissues. In addition to shape, movement, alteration, and integrity of tissues can also be observed in real time. However, the ultrasound measurement of shape is not sufficiently accurate if constant sound velocity is used. For example, information about cartilage thickness is altered by the change in sound velocity from cartilage degeneration. The aim of this study was to estimate sound velocity using surface echo reflectance. As a first stage for evaluating the clinical relevance of this method, the error of the cartilage thickness measurement was determined using collagenase-treated osteochondral plugs as a model of osteoarthritis (OA).
2. Material and methods

2.1 Preparation of cartilage samples

Osteochondral plugs (diameter 5 mm; n=30) were excised from a flat area of the trochlea femoris of frozen porcine knee joints. The osteochondral samples were removed from the subchondral bone using a microtome and the bottom was shaped parallel to the surface.

2.2 Enzyme treatment

The osteochondral samples (n=20) were digested in phosphate-buffered saline (PBS; Invitrogen Corp., Carlsbad, CA, USA) containing 30 U/ml collagenase type II at 37°C (Worthington Biochemical Corp., Lakewood, NJ, USA) for 4 and 9 hours (4 h and 9 h groups, respectively). Ten osteochondral samples in PBS alone at 37°C were used as controls (0 h group).

2.3 Ultrasound measurement

Figure 1 shows the ultrasound evaluation system for articular cartilage. The system consisted of a personal computer, a digital oscilloscope, a pulser/receiver, and a transducer (the ultrasonic probe) (XMS-310, Panametrics Japan Co., Ltd., Tokyo, Japan). The transducer emits and receives the ultrasound wave. A plane wave with a central frequency of 10 MHz is oscillated from the transducer. Reflected wave was received by the transducer and recorded by the oscilloscope. The sampling frequency was 500 MHz. The specimen was fixed on a stage in saline using bond, and the transducer was placed perpendicular to the surface of sample. Figure 2 shows a typical ultrasonic echogram demonstrating echo peaks from the surface and bottom of the specimen. Perpendicularity between the transducer and the cartilage surface was ensured by gently aligning the stage angle to obtain the maximum echo amplitude. The measurement was also performed with stainless steel as a specimen and the amplitude of echo from the surface was obtained.

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Fig. 1  (a) Schematic drawing of ultrasound measurement system. A small transducer was fixed over the measurement device. Measurement was performed in saline at room temperature and A-mode echogram was measured. (b) Ultrasound transducer (diameter: 2 mm; frequency: 10 MHz).
Fig. 2 Typical waveform of ultrasound echo. (a) Echoes from the surface and bottom of a specimen were recorded. The times to peak amplitude were recorded as $t_1$ and $t_2$. (b) Enlarged waveform of echo from surface of specimen.

The reflectance ratio was calculated from the equation:

$$R_c = \frac{A_c}{A_s} R_s$$  \hspace{1cm} (1)

where $A_s$ and $A_c$ are the maximum peak-to-peak echo amplitudes recorded from stainless steel and cartilage surfaces, respectively. $R_s$ and $R_c$ are the reflectance ratio of the steel–water and cartilage–water interfaces. In this study, $R_s = 0.936$ \(^\text{(6)}\) was used as the reflectance ratio of the steel–water interface.

2.4 Calculation of sound velocity

Sound velocity in articular cartilage was calculated from the reflectance ratio as follows:

$$c_c = \frac{1 + R_c}{1 - R_c} \frac{\rho_w}{\rho_c} c_w$$  \hspace{1cm} (2)
where $\rho_c$ and $\rho_w$ are the mass density of cartilage and water, and $c_c$ and $c_w$ are the sound velocities in cartilage and water, respectively. In this study, values of $\rho_c=1080$ kg/m$^3$ (7), $\rho_w=1000$ kg/m$^3$, and $c_w=1500$ m/s (6) were used.

2.5 Calculation of cartilage thickness

Cartilage thickness was calculated by multiplying interval time and sound velocity in the cartilage as follows:

$$H = c \cdot \frac{t_2 - t_1}{2}$$

where $H$ and $c$ are the cartilage thickness and sound velocity, respectively. Constant sound velocity (1600 m/s) (3) and calculated sound velocity were used as $c$.

2.6 Needle-probe measurement

The reference thickness of the cartilage samples was measured using the needle method (8), which is shown in Fig. 3. The specimen was placed on a holder and a sharp needle was pushed vertically into the cartilage at a constant displacement rate. A load cell detected the load signal. The load varied greatly when the needle made contact with the articular surface and cartilage–holder interface, and the displacement distance indicated thickness of the specimen.

The relative error of the ultrasonic measurement of cartilage thickness (measurement error) was determined according to the following equation:

$$e = \frac{H_U - H_N}{H_N} \cdot 100$$

where $e$ is the relative error, and $H_U$ and $H_N$ are the cartilage thickness from the ultrasound and needle methods, respectively.

![Fig. 3 Displacement and load signals for the determination of cartilage thickness during a needle test.](image-url)
2.7 Histological observation and biochemical examination

Each cartilage sample was divided into two after the ultrasonic evaluations; one part was used for histological analysis and the other for biochemical analysis. Histological samples were fixed in 10% formalin, decalcified in ethylenediaminetetraacetic acid, and embedded in paraffin. Sagittal sections (5 µm thick) were prepared from the center of the samples and stained with Safranin O.

Cartilage samples for biochemical examination were lyophilized overnight after measurement of wet weight. Dry weight was then measured, and water content of the cartilage (as a percentage) was determined according to the following equation: (wet weight–dry weight)/wet weight×100. Samples were then digested by adding 500 µl of 2.5% (w/v) actinase E solution prepared in 100 mM Tris-HCl buffer (pH 8.0), followed by incubation at 55°C for 16 h. Following digestion, samples were boiled for 10 min to inactivate the enzyme, and centrifuged at 10000 g for 15 min to obtain a clear supernatant free of insoluble materials. The amounts of chondroitin 4-sulfate, chondroitin 6-sulfate, and dermatan sulfate were evaluated to quantify proteoglycan content using high-performance liquid chromatography \(^9\). The amounts of unsaturated tetra- and hexasaccharide of hyaluronic acid were evaluated to quantify hyaluronic acid content \(^10\). Hydroxyproline content was evaluated to quantify collagen content \(^11\).

2.8 Statistical analysis

The Student’s \(t\)-test was used to determine the effects of enzyme treatment on reflectance ratio, sound velocity, and measurement error. Statistical significance was set at \(P<0.05\).

3. Results

Figure 4 shows the reflectance ratio decreasing exponentially with the enzyme treatment period. The calculated sound velocities in articular cartilage showed the same tendency as the reflectance ratio with significant differences between the 0 h, 4 h, and 9 h groups, as shown in Fig. 5. The relative errors of thickness measurements using constant sound velocity were –1 (SD 3)% for the 0 h group, 4 (SD 4)% for the 4 h group, and 8 (SD 4)% for the 9 h group; the respective errors using calculated sound velocity were 0 (SD 3)%, –2 (SD 3)%, and –1 (SD 2)% as shown in Fig. 6. Significant differences were found using constant sound velocity, whereas the errors using calculated sound velocity showed no significant difference between the groups.

The water contents were 82 (SD 1)% for the 0 h group, 83 (SD 1)% for the 4 h group, and 87 (SD 3)% for the 9 h group; the respective amounts of chondroitin sulfate were 2.5 (SD 0.2)%, 1.9 (SD 0.2)%, and 1.7 (SD 0.2)%, and those of hydroxyproline were 1.2 (SD 0.1)% 1.3 (SD 0.1)% and 1.3 (SD 0.2)% (Figs. 7–9). Representative sections of collagenase-digested cartilage stained with Safranin O are shown in Fig. 10. Homogeneity of Safranin O staining was increased with treatment time and the surface layer to a depth of 200 and 500 µm was not stained with Safranin O after 4 and 9 h, respectively.
Fig. 4  Changes in reflectance ratio of an ultrasound echo before (0 h), after 4 hours (4 h), and after 9 hours (9 h) of collagenase digestion. Mean values (SD) are shown for the 0 h (n=10), 4 h (n=10), and 9 h (n=10) groups.

Fig. 5  Changes in sound velocity calculated from equation (2) and from cartilage thickness measured with needle method and echo interval time before (0 h), after 4 hours (4 h), and after 9 hours (9 h) of collagenase digestion. Mean values (SD) are shown for the 0 h (n=10), 4 h (n=10), and 9 h (n=10) groups.

Fig. 6  Changes in error of thickness measurement before/after correction Mean values (SD) are shown for the 0 h (n=10), 4 h (n=10), and 9 h (n=10) groups. *Significant difference by Student’s t-test (P<0.05).
Fig. 7  Changes in water content in collagenase-digested articular cartilage before (0 h), after 4 hours (4 h), and after 9 hours (9 h) of collagenase digestion. Mean values (SD) are shown for the 0 h (n=10), 4 h (n=10), and 9 h (n=10) groups.

Fig. 8  Changes in chondroitin sulfate content in collagenase-digested articular cartilage before (0 h), after 4 hours (4 h), and after 9 hours (9 h) of collagenase digestion. Mean values (SD) are shown for the 0 h (n=10), 4 h (n=10), and 9 h (n=10) groups.

Fig. 9  Changes in hydroxyproline content in collagenase-digested articular cartilage before (0 h), after 4 hours (4 h), and after 9 hours (9 h) of collagenase digestion. Mean values (SD) are shown for the 0 h (n=10), 4 h (n=10), and 9 h (n=10) groups.
Fig. 10  Photomicrographs of pig articular cartilage before (a), after 4 hours (b), and after 9 hours (c) of collagenase digestion (Safranin O stain).

4. Discussion

Panula et al. reported that an increase in cartilage thickness occurred before macroscopic changes during early OA \((12)\). Although the accurate value of the increase is not clear, accurate thickness measurement might detect the early stage of OA. Yao and Seedhom indicated that the current ultrasound technique was not sufficiently accurate for measuring cartilage thickness in situ, because change in sound velocity was not considered in the calculation \((5)\). Myers et al. measured sound velocity in human femoral cartilage using ultrasound and histological measurement, and reported the velocity to be 1658 (SD 185) m/s for normal cartilage and 1581 (SD 148) m/s for OA cartilage \((13)\). These reports suggest a 10% order of error range of ultrasound thickness measurement for OA cartilage. In the present study, sound velocity in articular cartilage was calculated using echo reflectance from the surface \((R_c)\) and equation (2) assuming that the cartilage is homogeneous. The validity of this method was evaluated using collagenase-treated osteochondral plugs, and the treatment-dependent error was reduced from 7% to 2% in absolute value. The applicability of this method for the clinical measurement of cartilage thickness is discussed below.

Firstly, the present calculation still has a systematic error caused by adopting a constant mass density which is difficult to measure directly. The relationship between mass density and water content is shown as follows:

\[
\frac{\rho_w V_w}{W_w} = \frac{\rho_p (100 - V_w)}{100 - W_w} \quad (5)
\]

\[
\rho_c = \rho_w V_w + \rho_p (100 - V_w) \quad (6)
\]

where \(\rho_c\) is the mass density of cartilage, \(W_w\) is the water content by weight, \(V_w\) is the water content by volume, \(\rho_w\) is the mass density of water \(\left(\rho_w = 1000 \text{ kg/m}^3\right)\), and \(\rho_p\) is the mass density of the solid matrix of cartilage \(\left(\rho_p = 1470 \text{ kg/m}^3\right)\) \((14)\); the water content
increased from 82% to 87%. The mass density of cartilage decreased from 1080 to 1060 kg/m³ in the present study. As the decrease in mass density increases sound velocity by 2% in equation (2), it is suggested that the present method may still have a systematic error of –2% caused by the constant mass density.

Secondly, the effect of the layer structure of cartilage should also be considered. In articular cartilage, the orientation of collagen fibers is different for the superficial tangential zone (parallel to the cartilage surface) compared with the intermediate (random) and deep (perpendicular) zones. Patil et al. reported that sound velocity is higher in the deep zone than in the superficial zone (15). It suggested that sound velocity calculated using echo from the surface was lower than the average velocity of the whole cartilage. It is possible that the underestimation of velocity causes an underestimation of thickness.

Transection of the meniscus and/or ligaments, and intra-articular injection of a chemical substance such as papain or collagenase are generally performed to induce OA-like changes. In this study, however, collagenase-digested cartilage was used as a model of OA to control the degree of degeneration with high reproducibility. Wilson et al. reported an increase in water content, a decrease in proteoglycan content, and degradation of the collagen network during degeneration of OA cartilage (16). Additionally, Pritzker et al. reported cationic stain matrix depletion (Safranin O or toluidine blue) which progresses from the surface to the deep layers (17). As shown in Figs. 7–10, the same changes were observed in this study using collagenase-treated cartilage. However, additional studies using OA cartilage is required for more precise evaluation.

It is possible that reflection waveform changes shape with deformation of cartilage surface during OA progression. However, since there are many factors in cartilage surface like surface hydration layer, it is difficult to discuss using simple model. The changes in cartilage surface during OA progression and effect of the changes on reflection waveform is currently under investigation.

In summary, the validity of the correction method for ultrasound measurement of cartilage thickness was evaluated using collagenase-treated osteochondral plugs, where sound velocity in articular cartilage was calculated from reflectance ratios. The collagenase treatment-dependent error was reduced from 7% to 2% in absolute value, which suggests the applicability of the correction method for ultrasound measurement. Studies of several factors such as the difference in surface and layer structure between the enzyme-treated model and degenerated cartilage are recommended for more precise evaluation.

References


