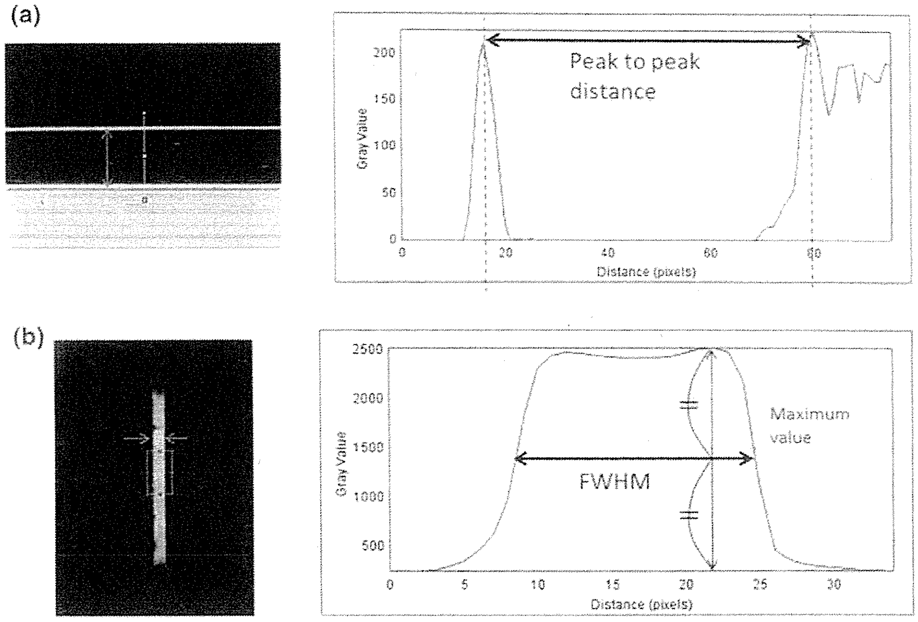

Fig. 2 Thickness measurement of agar phantom: **a** pulse-echo method by use of peak-to-peak distance of a profile curve; **b** full width at half maximum (*FWHM*) for MRI. The unit of gray value in the figure, means arbitrary unit

accuracy. Each measurement was performed five times, and the mean values of these measurements were used in this study.

2.7 Statistics

Statistical analysis was performed with use of the Wilcoxon signed-rank test. Statistical significance was defined by $p < 0.05$. Statistics software (Statview, version 5; SAS Institute, Cary, NC, USA) was used for all analyses.

3 Results

3.1 Measurements of thickness of specimens

The mean thicknesses of the agar phantoms obtained with MRI and laser measurements were 5.71 ± 0.33 and 5.70 ± 0.33 mm, respectively. Thus, there was no significant difference between MRI and laser measurements ($p < 0.05$). The mean thicknesses of the porcine knee cartilage specimens obtained with MRI and pulse-echo ultrasound measurement were 2.63 ± 0.92 and 2.56 ± 0.91 mm, respectively.

3.2 SOS measurements in specimens

The SOS in agar phantoms without glycerin was measured by transmission ultrasound and showed a constant value (approximately 1336 m/s, at 18.4 °C). When the SOS in the agar phantoms that included different concentrations of glycerin was measured, the SOS values were increased with increasing glycerin concentrations. The measured SOS values increased by 36.38 m/s per 5 % of added glycerin when measured with transmission ultrasound, and increased by an 35.67 m/s per 5 % of added glycerin when measured with the combined measurement method of MRI and pulse-echo ultrasound. Figure 3 shows the relationship between the SOS measured in agar phantoms that included various concentrations of glycerin for the combined

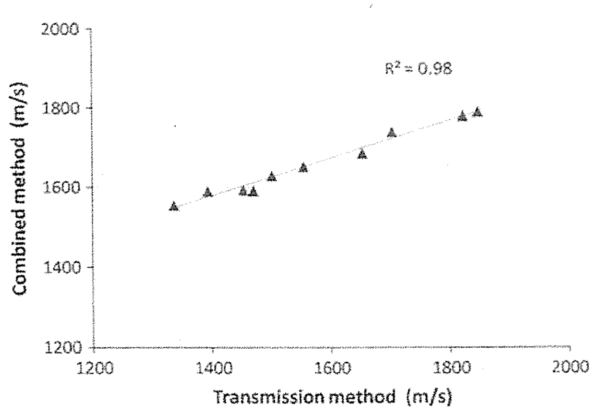

Fig. 3 Correlation of SOS measurements in agar phantom by use of transmission method and combined method. Transmission ultrasound measurement and combined method are strongly correlated (correlation coefficient = 0.98). The high accuracy of the combined method was confirmed. The unit of speed of sound in the figure, "m/s", means meters/second

method and the transmission ultrasound methods. A significant linear correlation was observed between the results based on the combined method and those based on transmission ultrasound alone ($R^2 = 0.98$, $p < 0.05$).

The mean SOS in the porcine knee cartilage specimens which was calculated by the combined method was 1575 ± 119 m/s.

4 Discussion

The present study demonstrated the accuracy of our proposed method of combined MRI and pulse-echo ultrasound measurements for the assessment of SOS in cartilage. The accuracy of SOS measurements by use of the combined method depends on the accuracy of the cartilage thickness measurements. It is known that pulse-echo ultrasound provides very high spatial resolution. Although Eckstein et al. [12, 13] reported an underestimation of cartilage thickness measurements when using a 1.5T MRI system, and suggested that the low signal-to-noise (SNR) and low spatial resolution of the MR images were possible reasons for the inaccuracies, our thickness measurements of agar phantoms with use of a 3T MRI system indicated that the thickness of these objects could be measured accurately, with an error of less than a single pixel. The smallest isotropic voxel size of MR images is typically 0.6 mm in clinical use, but improvements in compression sensing technology are expected to lower this limit in the near future.

The SOS measurements by use of the combined method and the ultrasound transmission method showed a high correlation in the agar phantoms. There was no significant difference in measurements by these two methods, and measurements with the combined method were obtained correctly. The reason for the difference in SOS between the transmission method and the combined method was that the transmission method was affected by the temperature. An advantage of the SOS calculated from the combined method is that it does not depend on the temperature. Theoretically, the SOS of the transmission method and that of the combined method are in a one-to-one relationship at a temperature of 37 °C. Furthermore, the SOS in porcine knee cartilage specimens measured by the combined method in our study was similar to that of previous studies (Table 2) [14, 15]. In practice, as no biochemical and histological analysis was performed, the degree of cartilage degeneration is not known.

The cartilage thickness measurement necessary for calculations of SOS in tissue were based on morphological images, but the SOS values obtained provide only narrow area information. Using two modalities to obtain the SOS may be difficult in clinical practice, but there is also an advantage, namely, that of enabling measurements of elasticity in tissue in vivo, which was previously assumed to be impossible. In any case, when using the two measurement modalities, real-time virtual sonography provides agreement of the measurement region of the cartilage. Thus, the SOS measurements obtained by the combined method allow the elasticity in living tissue such as cartilage to be evaluated, which cannot be done with the use of conventional elastography based on speckle tracking. Conventional elastography has been used as a non-invasive method for evaluating elasticity in several types of tissue; however, its application to cartilage is difficult, because cartilage itself does not have any echo signals [6, 7]. For example, our method may be useful for follow-up examinations of osteoarthritis patients who have received grafts of regenerated cartilage.

Table 2 Summary of reported speed of sound measurements in articular cartilage

Author (year)	Articular cartilage specimen	Speed of sound (m/s)
Myers et al. [14]	Human femoral condyle	1658 ± 185
Suh et al. [1]	Human femoral condyle	Normal 1735 ± 35 PG-depleted 1598 ± 28
Joiner et al. [15]	Bovine femoral condyle	1666 ± 16
Combined method	Porcine femoral condyle	1575 ± 119

5 Conclusions

The present study demonstrated the accuracy of the proposed measurement method that combines MRI and pulse-echo ultrasound measurements for assessment of the SOS in cartilage. The use of this method as a new non-invasive diagnostic tool may be expected in the future.

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Conflict of interest All authors certify that there is no financial conflict related to the present subject matter or to any materials discussed in this manuscript.

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