Can ultrasound predict histological findings in regenerated cartilage?

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Objective. To evaluate regenerated articular cartilage quantitatively by introducing an ultrasonic probe into the knee joint under arthroscopy and analysing the A-mode echogram by means of wavelet transformation.

Methods. Three experimental rabbit models (spontaneous repair model, large cartilage defect model, treatment model) were examined using our ultrasonic evaluation system and a histological grading scale. From resulting wavelet map, the percentage of maximum magnitude was selected as the quantitative index of the ultrasonic evaluation system.

Results. The percentage maximum magnitude in the spontaneous repair model was 61.1%, that in the large defect model was 29.8% and that in the treatment model was 36.3%. There was modest correlation between the percentage maximum magnitude and the histological grading scale (r = −0.594)

Conclusion. Our findings indicate that ultrasound analysis can predict the microstructure of regenerated cartilage.

Numerous attempts to induce the healing of large articular cartilage defects have been made. Each treatment has its own strong and weak points, and it remains difficult to choose among treatments. There are no quantitative non-invasive methods to evaluate the regenerated cartilage clinically [1]. Generally, arthroscopy can be performed to evaluate regenerated cartilage. However, although arthroscopy can identify cartilage surface changes, it cannot reveal the microstructure of the regenerated cartilage. Brismar et al. [2] reported on observer reliability in arthroscopic grading of osteoarthritis of the knee, and concluded that observer-specific disagreement was evident both within and between observers and that arthroscopic grading of early osteoarthritic lesions was inexact. Therefore, arthroscopic judgement cannot exclude the potential for observer bias in the evaluation of the cartilage surface. For the strict assessment of regenerated cartilage, histological and biochemical analyses are performed from experimental and clinical aspects. However, these analyses require collection of the regenerated tissue, and the biopsy could possibly induce the progression of osteoarthritis. Therefore, these evaluation methods should be avoided.

We developed an intra-articular ultrasonic probe and a new evaluation system for articular cartilage in the human knee, and revealed that this system was able to quantitatively evaluate cartilage degeneration [3, 4]. However, it remains to be shown whether this system can accurately evaluate regenerated cartilage. The purpose of this study was to determine the efficacy of our system for evaluating regenerated cartilage. To this end, we quantitatively evaluated regenerated cartilage using experimental rabbit models.

Materials and methods

The experimental rabbit models used in this study consisted of three types of cartilage defect: the spontaneous repair model as a positive control, the large cartilage defect model as a negative control, and the treatment model, consisting of a large cartilage defect covered with periosteum. Six adolescent Japanese white rabbits (2.3–2.5 kg) were used for the spontaneous repair model, and 6 adult rabbits (4.2–4.5 kg) were used for each of the large cartilage defect and treatment models. This study was approved by the Nara Medical University Ethics Committee.

Experimental models

For the spontaneous repair model, adolescent rabbits were anaesthetized and an anteromedial arthrotomy was performed in one knee. The patella was dislocated laterally and two defects, 3 mm in diameter and 3 mm in depth, penetrating the subchondral bone plate, were created on the patellar groove of the femur. The defects were washed with saline but left without any further treatment. The wound was then closed in layers. As a control, the procedure was not performed in the other knee.

For the large cartilage defect model, adult rabbits were operated on as described above. The articular cartilage was resected with a chisel to make a 5 mm diameter defect down to the subchondral bone. The defects were washed with saline and the wound was then closed in layers.

For the treatment model, The adult rabbits were operated on as described for the large cartilage defect model. A periosteal graft was obtained from the medial side of the proximal end of the tibia, transplanted to the defect with the cambium layer facing up into the joint, and fixed with 4–0 nylon sutures. The wound was then closed in layers.

The animals were returned to their cages and allowed to move freely without joint immobilization. Two adolescent rabbits were excluded from this series: one died 3 days after the operation and another showed signs of infection 6 days after the operation. The rabbits were killed at 12 weeks with an overdose of phenobarbital.
sodium salt. The knee joints were opened and dissected free from all soft tissues, and the tibia was removed. The distal femur was cut proximally to the patellofemoral joint and the cartilage samples were taken.

**Ultrasonic evaluation**

Ultrasonic examination was made in saline using a transducer and pulser receiver (Panametrics Japan, Tokyo, Japan) (Fig. 1A). The reflex echogram from the cartilage was transformed into a wavelet map using wavelet transformation. For the mother wavelet function, Gabor’s function was selected. The maximum magnitude was selected as a quantitative index on the wavelet map (Fig. 1B).

The cartilage samples from the three models were evaluated in saline and the transducer was placed over the samples. The results obtained for the ultrasonic evaluation were averaged values of five measurements. For the spontaneous repair model and the control, the measurement points were the centre part of the defect, and for the large cartilage defect and treatment models, the measurement points were the centre and 4 points 1mm up, down, left and right of the centre. The percentage of the maximum magnitude (measurement area/intact cartilage of control) was used as the quantitative index of cartilage repair.

**Histological evaluation and scoring**

After ultrasonic evaluation, cartilage samples were fixed in 10% formalin, decalcified in EDTA and embedded in paraffin. Sagittal sections of 5μm thickness were prepared from the centre of the defect area, and stained with haematoxylin and eosin, toluidine blue O and safranin O. Each section was graded using the histological scale described by Wakitani et al. [5]. The total score was low for cases of good cartilage regeneration.

**Statistical analysis**

Differences in ultrasonic data were analysed with the non-parametric Mann-Whitney U-test. Pearson correlations were calculated to determine the association between the ultrasonic data and the histological data. The significance level was set at $P < 0.05$.

**Results**

**Ultrasonic findings**

The percentage of the maximum magnitude in the spontaneous repair model was 61.1%, that in the large defect model was 29.8% and that in the treatment model was 36.3% (Fig. 2A). The percentage maximum magnitude in the spontaneous repair model was higher than those in the large defect and treatment models ($P < 0.01$). However, no significant difference in the percentage maximum magnitude between the large defect and treatment models was found ($P = 0.51$).

**Histological findings**

The defect area in the spontaneous repair model was filled with repaired tissue that consisted of three types (fibrous tissue, fibrocartilage and hyaline-like cartilage). Fibrous tissue and fibrocartilage were seen in the superficial layer of the repaired tissue. In the superficial layer, safranin O staining was less intense. Hyaline-like cartilage had formed in the deeper layers (70–80% of the total layer). In the deeper layers, chondroid cells with round nuclei were observed in an extracellular matrix with normal or nearly normal safranin O staining. The defect area in the large defect model was filled with fibrous tissue. All the sections of the large defect model had no fibrocartilage or hyaline-like cartilage and were not stained with safranin O. The defect area in the treatment model was filled with hyperplastic tissue. Fibrous tissue was seen in the superficial and middle layers of the repaired tissue. In the deeper layers, chondroid cells with round nuclei were observed in an extracellular matrix with normal or nearly normal safranin O staining.

**Histological grading scores and correlation between the ultrasonic data and the histological scale**

The histological grading score was low for cases of good cartilage regeneration. The score of the spontaneous repair model was 4.6 points (range 2–7 points). The score of the large cartilage defect model was 10.5 points (range 9–12 points). The score of the treatment model was 7.2 points (range 4–11 points). There was modest correlation between the percentage maximum magnitude for the ultrasonic examination and the score on the histological grading scale for the results of all the measurements (Fig. 2B).

**Discussion**

From the results of this study, ultrasound analysis offers promise as a non-invasive method of evaluating regenerated articular cartilage. Three experimental models were evaluated quantitatively using the percentage of maximum magnitude. A modest correlation between the percentage of maximum magnitude and the histological grading score was found from the results of all the measurements.

Several researchers have reported a relationship between the acoustic properties and matrix degeneration of cartilage using enzyme-induced degenerated cartilage samples in vitro. Joiner et al. [6] revealed that matrix degeneration and proteoglycan loss resulted in a decrease in the speed of sound and an increase in the frequency-dependent attenuation. Töyäräs et al. [7] investigated enzyme-induced degenerated cartilage using a scanning ultrasound system that enables the generation of A-, B-, C-, D-, F- and M-mode images. These high-resolution images suggested that ultrasound could be used for the evaluation of spatial changes in the cartilage properties. Therefore, ultrasound could provide valuable information concerning structural and material abnormalities in articular cartilage. Our evaluation method using wavelet transformation is highly reproducible and the ultrasonic probe used in the evaluation is so small that it should be useful for the clinical assessment of articular cartilage condition in arthroscopy.

As new cartilage treatment methods come into use, there will be a requirement for non-invasive quantitative evaluation of the regenerated cartilage. A reliable evaluation method, if established, would help answer the important clinical question of whether repair tissue is mainly hyaline cartilage or mainly fibrocartilage. In our study, all hyaline cartilage (control sites) had a higher maximum magnitude than the repaired tissue, and imperfect regenerated cartilage had a reduced maximum magnitude, even if fibrous tissue and fibrocartilage were seen only in the superficial layer of the repaired tissue in the case of the spontaneous repair model. However, we could not judge the borderline of the two types of regenerated tissue by the percentage of maximum magnitude, and further detailed research on our evaluation method is now required.

In conclusion, ultrasonic evaluation using wavelet maps can predict the histological findings of regenerated cartilage using three experimental models. This evaluation system should contribute greatly to orthopaedics and rheumatology, and assist in the progress of cartilage regeneration.
Fig. 1. (A) Schematic illustration of ultrasonic examination. Experimental measurements were performed in saline at room temperature and clinical measurements were performed with an ultrasonic probe under arthroscopy. (B) A-mode echogram and wavelet map of intact articular cartilage. Wavelet maps have two peaks of magnitude: the left peak is the reflex echo from the cartilage and the right peak is that from the subchondral bone. The quantitative parameters of the cartilage are shown as the maximum magnitude.
No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

**References**


**Fig. 2.** (A) Mean percentage of maximum magnitude for the three experimental models. The error bars represent the standard deviation of each group. $P < 0.05$ by non-parametric Mann–Whitney U-test analysis. (B) Histological grading score from microscopic findings vs percentage of maximum magnitude from ultrasonic examination ($r = -0.594$; $Y = -8.89x + 11.19$; $P = 0.0057$).