INTRODUCTION
Numerous attempts to induce the healing of large articular cartilage defects have been made, including drilling, abrasion chondroplasty, microfracture, periosteal grafting, mosaicplasty and cultured autologous chondrocyte transplantation. Each treatment has its own strong and weak points, and the efficacies of all the current techniques are still being evaluated by basic scientific research and clinical trials. In particular, with regard to the new treatments for large cartilage defects, it is of great concern to both orthopedic surgeons and researchers whether the repair tissue is hyaline cartilage or fibrocartilage. However, without a biopsy (causing damage), there are no clinical methods for distinguishing between these two types of tissue.

We developed a new evaluation system for articular cartilage and revealed that this system was able to quantitatively evaluate cartilage degeneration and cartilage repair. However, it remains to be shown whether this system can accurately evaluate the histological findings of regenerated cartilage. The purpose of this study was to determine the borderline between the two types of regenerated tissue using the evaluation system.

MATERIALS AND METHODS
The rabbit experimental models used in this study comprised of four types of cartilage defect: the spontaneous repair model (group S; cartilage defect, 3 mm in diameter) as a positive control, the large cartilage defect model (group L; cartilage defect, 5 mm in diameter) as a negative control, the periosteal graft model (group P; a large cartilage defect covered with periosteum), and the tissue-engineered cartilage regeneration model (group T; a large cartilage defect that bone marrow-derived mesenchymal stem cells (MSCs) in a three-dimensional poly-lactic glycolic acid (3DPLGA) scaffold composite went into).

The ultrasonic examination was made in saline using a transducer and pulsed receiver [1]. The transducer was 3 mm in diameter and 3 mm in length, and sent and received a flat ultrasonic wave of 10 MHz center frequency. 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. As quantitative indices on the wavelet map, the maximum magnitude was selected. This index was calculated automatically by a personal computer. In this study, the % maximum magnitude (measurement area / intact cartilage) was used as the quantitative index of cartilage repair, because of the variety in rabbit age.

After ultrasonic evaluation, Histological evaluation and scoring were performed. Cartilage samples were stained with hematoxylin and eosin, toluidine blue and Safranin-O. Sections from each model were examined and scored independently. Each sample was graded using the histological scale described by Caplan et al., which was composed of six categories and assigned a score ranging from 0 to 16 points [2]. The total score was high for cases of good cartilage regeneration.

RESULTS
In the histological findings, the defect area in group S 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. Hyaline-like cartilage had formed in the deeper layers (from 70 to 80% of the total layer) (Fig. 1A, 1B). The defect area in group L was filled with fibrous tissue. None of the sections of the large defect model had any fibrocartilage or hyaline-like cartilage (Fig. 1C, 1D). The defect area in group P 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 (Fig. 1E, 1F). The defect area in group T was filled with hyaline-like cartilage and chondroid cells lay in uniform with the palisade (Fig. 1G, 1H).

The histological score was 9.7 points in group S, 4.2 points in Group L, 6.3 points in Group P and 10.7 points in Group T. Significant differences in the score were seen between S and L (P = 0.0002), S and P (P = 0.0058), L and T (P = 0.0007), and P and T (P = 0.0002) (Fig. 2A).

DISCUSSION
The results of this study indicate that ultrasound analysis is promising as a non-invasive method for evaluating the microstructure of regenerated articular cartilage, as assessed using four experimental models. The ultrasonic results showed that all the hyaline-like cartilage had a high %MM of more than 60%. Therefore, we could define the borderline between the two types of regenerated cartilage by the %MM. A significant difference was detected between groups P and T. This result suggests that treatment of critical cartilage defects with the MSCs/3D-PLGA scaffold composite was more effective than treatment with a periosteal graft.

REFERENCES