Which cartilage is regenerated, hyaline cartilage or fibrocartilage? Non-invasive ultrasonic evaluation of tissue-engineered cartilage


Objective. To investigate ultrasonic evaluation methods for detecting whether the repair tissue is hyaline cartilage or fibrocartilage in new cartilage regeneration therapy. 

Methods. We examined four experimental rabbit models: a spontaneous repair model (group S), a large cartilage defect model (group L), a periosteal graft model (group P) and a tissue-engineered cartilage regeneration model (group T). From the resulting ultrasonic evaluation, we used %MM (the maximum magnitude of the measurement area divided by that of the intact cartilage) as a quantitative index of cartilage regeneration. The results of the ultrasonic evaluation were compared with the histological findings and histological score.

Results. The %MM values were 61.1 ± 16.5% in group S, 29.8 ± 15.1% in group L, 36.3 ± 18.3% in group P and 76.5 ± 18.7% in group T. The results showed a strong similarity to the histological scoring.

Conclusion. The ultrasonic examination showed that all the hyaline-like cartilage in groups S and T had a high %MM (more than 60%). Therefore, we could define the borderline between the two types of regenerated cartilage by the %MM.

KEY WORDS: Ultrasound, Articular cartilage, Evaluation, Hyaline cartilage, Tissue engineering.

Numerous experimental and clinical 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 in 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 orthopaedic surgeons and researchers whether the repair tissue is hyaline cartilage or fibrocartilage [1]. However, without a biopsy (which causes 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 [2, 3]. 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 this evaluation system.

Materials and methods

The rabbit experimental models used in this study comprised four types of cartilage defect: the spontaneous repair model (group S) as a positive control, the large cartilage defect model (group L) as a negative control, the periosteal graft model (group P), consisting of a large cartilage defect covered with periostem, and the tissue-engineered cartilage regeneration model (group T), consisting of a large cartilage defect into which bone marrow-derived mesenchymal stem cells (MSCs) in a three-dimensional polylactic glycolic acid (3D-PLGA) scaffold composite were introduced. Four adolescent Japanese white rabbits (2.3–2.5 kg) were used for the spontaneous repair model, and six adult rabbits (3.4–4.5 kg) were used for each of the other three models. This study was approved by the Nara Medical University Ethics Committee.

In the spontaneous repair model, adolescent rabbits were anaesthetized with a mixture of ketamine (50 mg/ml) and xylazine (20 mg/ml) at a ratio of 2:1, in a dose of 1 ml/kg injected into the gluteal muscle. An anteromedial arthrotomy was performed in one knee with the joint positioned at maximum flexion. 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 patella groove of the femur using a stainless steel reamer. The defects were washed with saline and dried with a swab to remove any debris, but left without any further treatment. The wound was then closed in layers with 2-0 vicryl sutures. The procedure was not performed in the other knee as a control.

In the large cartilage defect model, adult rabbits were anaesthetized as described for the spontaneous repair model. The patella was dislocated laterally and articular cartilage was resected with a chisel in a 5 mm diameter defect down to the subchondral bone. The defects were washed with saline and dried with a swab to....
remove any debris, but left without any further treatment. The wound was then closed in layers with 2–0 vicryl sutures. In the periosteal graft model, adult rabbits were anaesthetized and 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 upwards into the joint and fixed with 4–0 nylon sutures. The wound was then closed in layers with 2–0 vicryl sutures.

The 3D-PLGA scaffold used for the tissue-engineered cartilage regeneration model has been described previously [4]. This scaffold has numerous cylindrical boreholes, and within the scaffold the cells lie in a uniform array at the palisade. As the cultured cells used in this study, bone marrow-derived MSCs were prepared. Bone marrow cells were aspirated from the humeral head of each rabbit and primary culture was performed in T75 flasks (Costar, Cambridge, MA, USA). The primary culture was maintained under standard culture conditions for 2 weeks and then the cultured MSCs were seeded into the 3D-PLGA scaffold at 10^6 cells/cm² (MSCs/3D-PLGA scaffold composite). The MSCs/3D-PLGA scaffold composite was transplanted into the defect and fixed using a fibrin sealant (Tissiefax; Baxter, Vienna, Austria). The wound was then closed in layers with 2–0 vicryl sutures.

The rabbits were returned to their cages and allowed to move freely without joint immobilization. The rabbits were killed at 12 weeks with an overdose of phenobarbital sodium salt. All 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 cartilage samples were taken.

**Ultrasonic evaluation**

The ultrasonic evaluation method has been described elsewhere [2, 3]. Briefly, the ultrasonic examination was made in saline using a transducer and pulsar receiver (Panametrics Japan, Tokyo, Japan). The transducer was 3 mm in diameter and 3 mm in length, and sent and received a flat ultrasonic wave of 10 MHz centre frequency. The reflex echogram from the cartilage was transformed into a wavelet map using wavelet transformation. As a quantitative index of the wavelet map, the maximum magnitude was selected. The ultrasonic evaluation was performed by a coresearcher (MT) who had no knowledge of the study groups (and was thus a blind observer). The results obtained for the ultrasonic evaluation were averages of five measurements. For the spontaneous repair model, the measurement points were in the centre part of the cartilage defect, while for the other three models the measurement points were the centre and four points 1 mm above, below, left and right of the centre. The percentage maximum magnitude (%MM: the maximum magnitude of the measurement area divided by that of the intact cartilage) was used as the quantitative index of cartilage regeneration.

**Histological evaluation and scoring**

After the ultrasonic evaluation, the cartilage samples were fixed in 10% formalin, decalcified in EDTA (ethylenediamine tetraacetate) and then embedded in paraffin. Sagittal sections of 5 μm thickness were prepared from the centre of the defect area, and stained with safranin-O. Each section was graded using the thickness were prepared from the centre of the defect area, and 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 cartilage samples were taken.

**Statistical analysis**

Differences were analysed with the non-parametric Mann–Whitney U test. The significance level was set at P < 0.05.

**Results**

**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 (70–80% of the total layer) (Fig. 1A and B). 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 and D). 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 and F). The defect area in group T was filled with hyaline-like cartilage and chondroid cells lay in uniform with the palisade (Fig. 1G and H).

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), between S and P (P = 0.0058), between L and T (P = 0.0007), and between P and T (P = 0.0002) (Fig. 2A).

**Ultrasonographic findings**

The %MM results showed a strong similarity to the histological scoring. The %MM values were 61.1±16.5% in group S, 29.8±15.1% in group L, 36.3±18.5% in group P and 76.5±8.7% in group T. Significant differences in %MM were seen between groups S and L (P = 0.003), S and P (P = 0.021), L and T (P = 0.007), and P and T (P = 0.004). The average %MM in group T was the highest among all the groups. However, there was no significant difference in the %MM between S and T (P = 0.13) (Fig. 2B).

**Discussion**

The results of this study indicate that ultrasound analysis is promising as a non-invasive method for evaluating the micro-
structure of regenerated articular cartilage, as assessed using four experimental models. The ultrasonic results showed that all the hyaline-like cartilage had a high %MM (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. Moreover, ultrasonic evaluation could define the borderline between the two types of regenerated tissue, using the %MM. Therefore, this evaluation system should contribute greatly to the progress of cartilage regeneration. Further detailed research on new treatments for large cartilage defects is now required.

In conclusion, ultrasonic evaluation using a wavelet map can support the determination of the efficacy of new treatment methods for large cartilage defects. The evaluation system is suitable for clinical use under arthroscopy. This evaluation successfully predicted the histological findings of regenerated cartilage using four experimental models. We believe our findings offer the potential for standardized evaluation as an adjunct to further research in this field, which will lead to a reliable method for the quantification of articular cartilage treatments.

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References