INTRODUCTION

Although numerous methods for regenerating articular cartilage have been investigated, the regenerated tissue showed various histological findings from hyaline-like cartilage to fibrous tissue. Without biopsy, we are unable to know whether the cartilage regeneration method was histologically successful or not.

We developed a new evaluation system for articular cartilage and revealed that this system was able to quantitatively evaluate cartilage degeneration. However, it remains to be shown whether this system can accurately judge the success or failure of the cartilage regeneration procedure. The purpose of this study was to investigate the usefulness of ultrasonic judgment of the cartilage regeneration procedure. Using our system, we quantitatively evaluated tissue-engineered cartilage in rabbit cartilage defects then performed histological analysis of the tissue-engineered cartilage. The specimens were retrospectively divided into two groups on the basis of the histological findings then we investigated whether significant differences in ultrasonic analysis could be found between the two.

MATERIALS AND METHODS

Twenty-five adult rabbits were used. The rabbits were anesthetized and an anteromedial arthrotomy was performed in one knee. The patella was dislocated laterally and the articular cartilage was resected with a chisel to form a 6 mm-diameter defect down to the subchondral bone. The defect was washed with saline and dried with a swab to remove any debris then the stem cell/scaffold complex was transplanted onto the defect. It was fixed using a fibrin sealant and the wound was then closed in layers with 2-0 vicryl sutures.

Ultrasonic evaluation

The ultrasonic examinations were conducted in saline using a transducer and pulser receiver (Fig. 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. Reflex echograms from the cartilage were transformed into a wavelet map using wavelet transformation. For the mother wavelet function, Gabor’s function was selected and as the quantitative index on the wavelet map, the maximum magnitude (MM) was selected.

![Fig. 1. Schematic illustration of the articular cartilage analysis and measurement methods of cartilage samples. A reflex echo of articular cartilage (upper) and a wavelet map (lower) are shown on the right. The maximum magnitude is indicated by the gray scale.](Image)

Histological evaluation

After ultrasonic evaluation, histological assessment was performed by a consultant histopathologist. Cartilage samples were examined under direct light-microscopy and divided into two group (group H: hyaline-like cartilage group; and group F: fibrous tissue group) by the histopathologist.

Histological scoring

The sections were scored by an orthopaedic surgeon under blinded conditions according to a semiquantitative histologic grading scale described by Caplan et al. A high total score represented good cartilage regeneration.

RESULTS

From the histological findings, samples were divided into two groups; 16 samples were hyaline-like cartilage (group H) and 9 were fibrous tissue (group F) (Fig. 2). These histological findings show that the success rate of the tissue-engineered cartilage procedure was 64.0%.

![Fig. 2. Photomicrographs of tissue-engineered cartilage on rabbit cartilage defects (Safranin-O fast green). The upper sample indicates good cartilage regeneration as hyaline cartilage (group H) and the lower sample is covered with fibrous tissue (group F).](Image)

In the ultrasonic findings, the MM was 0.95 ± 0.36, and according to the groups, it was 1.11 ± 0.32 for group H and 0.65 ± 0.18 for group F. Significant difference was found between groups H and F (Fig. 3A). The semiquantitative histologic grading scale score was 7.76 ± 4.18, and according to the groups, it was 10.4 ± 2.6 for group H and 3.11 ± 1.27 for group F. Significant difference was found between groups H and F (Fig. 3B). There was a modest correlation between the MM and the histologic grading scale scores ($R^2 = 0.43$).

![Fig. 3. (A) Mean maximum magnitude of the two groups according to the ultrasonic findings. (B) Mean histological grading score of the two groups according to the semiquantitative histologic grading scale. The error bars represent the standard deviation of each group. * $P<0.05$ by non-parametric Mann-Whitney U-test analysis.](Image)

DISCUSSION

The results of this study show that ultrasound analysis is a promising non-invasive method of evaluating regenerated articular cartilage. The tissue-engineered cartilage was evaluated quantitatively using MM and a semiquantitative histologic grading scale. Significant differences in MM were found between groups H and F, and a modest correlation was found between the MM and histological grading scores of the overall results from all measurements. Therefore, this ultrasonic cartilage evaluation system can accurately judge the success or failure of the cartilage regeneration procedure.

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