Meniscal Repair Using Biodegradable Gelatin Hydrogel Incorporating Fibroblast Growth Factor 2: Experimental Study in Rabbits
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INTRODUCTION
The meniscus has a low healing potential, and in some cases, primary repair is unsuccessful. Several growth factors have been proven to be effective in enhancing meniscal tissue regeneration. However, the use of growth factors alone is not always successful, as the half-lives of many growth factors are too short to sustain biological activity. To correct for this problem, we developed a controlled release system using gelatin hydrogel. The growth factors are immobilized in gelatin hydrogel through a physicochemical interaction with gelatin molecules, and the immobilized growth factors are released from the hydrogel as a result of hydrogel degradation. Fibroblast growth factor 2 (FGF 2) is known to stimulate fibroblast proliferation and enhance collagen synthesis.

MATERIALS AND METHODS
Gelatin hydrogels were prepared by chemical cross-linking of aqueous gelatin solution with glutaraldehyde as reported previously. Gelatin hydrogel sheets were cut into small rectangles (2×3mm), and 2 μL of FGF 2 solution (1 μg/μL) was added onto the freeze-dried gelatin hydrogel sheets and left for 1 hour to allow it to impregnate the hydrogel. Similarly, gelatin hydrogel with double-distilled water (DDW) was prepared as a control.

RESULTS
In a blinded fashion, horizontal tears were divided into 2 groups according to treatment as follows: FGF(+) group, tears were filled with gelatin hydrogel sheets incorporating FGF2; FGF(-) group, tears were filled with gelatin hydrogel sheets containing DDW.

The surgically treated meniscus was taken from the knee joint and evaluated at 2 and 4 weeks after surgery. Cell density, number of PCNA-positive and TUNEL-positive cells in the FGF(+) and FGF(-) groups were measured adjacent to the tear, as shown in Fig. 2. In both groups, we tested 2 "outer zones" adjacent to the peripheral rim of the meniscus, and 2 "inner zones" adjacent to the edge of the tear.

Reparative tissue was evaluated by semi-quantitative scoring as follows. 4 points: the tear was regenerated by normal meniscal tissue. 3 points: the tear was entirely closed off with fibrous tissue. 2 points: the tear was not entirely closed off, fibrous tissue infiltrated into the inner zone. 1 point: the tear was not entirely closed off, fibrous tissue infiltrated into the outer zone. 0 points: no evidence of fibroblast proliferation. Statistical analyses of the data were carried out using Student’s t-test. Differences of P < 0.05 were considered to be significant.

The number of TUNEL-positive cells in the outer zone was significantly lower in the FGF(+) group (105.7±49.0) than in the FGF(-) group (168.8±44.6), at 2 weeks. At 4 weeks, it was significantly lower in the FGF(+) group (56.3±37.7) than in the FGF(-) group (162.8±49.4). In the inner zone, it showed no difference between 2 groups (Fig. 5).

DISCUSSION
Our results suggested that the gelatin hydrogels incorporating FGF 2 stimulated proliferation and inhibited DNA fragmentation of rabbit meniscal cells, primarily influencing meniscal cells in the outer zone. A reparative change was observed in the outer zone in both the FGF(+) and FGF(-) groups. On the other hand, in the FGF(-) group, reparative changes in the inner zone were not observed. We speculate that FGF 2 activated two processes: 1. meniscal cells derived from the surrounding meniscus were migrated into the tear; 2. meniscal cells in the outer zone were migrated into the tear. FGF 2 further stimulated these migrated cells, and the tear was closed in the inner zone.

It is likely that FGF 2 enhanced the biological activities of the meniscal cells for meniscal tissue regeneration. Further investigation is needed to evaluate long-term outcome and extracellular matrix, although we believed that FGF 2 incorporated gelatin hydrogels is an effective biomaterial for meniscal repair.