REGENERATION OF FULL-THICKNESS DEFECTS OF ARTICULAR CARTILAGE IN RABBITS USING FGF-2 AND FIBRIN SEALANT

+*Ishii, I; *Mizuta, H; *Kudo, S; *Takagi, K; **Hiraki, Y

+ Department of Orthopedic Surgery, Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Japan.
+81-96-373-5226, Fax: +81-96-373-5228, 030r5106@med.stud.kumamoto-u.ac.jp

INTRODUCTION
We previously showed that a continuous administration of fibroblast growth factor (FGF)-2 with an osmotic delivery system promoted proliferation and migration of mesenchymal cells and induced a successful cartilage repair response in 5-mm full-thickness articular cartilage defects in rabbits. The purpose of this study is to develop an FGF-2 delivery system for clinical use in cartilage repair. Therefore, the following experiment was designed to quantify diffusive release of FGF-2 from FGF-2-incorporating fibrin sealant (FS) in vitro and to clarify the bioactivity of FGF-2-incorporating FS both in vitro and in vivo.

METHODS
EXPERIMENT-1: Firstly, 0.1ml of fibrinogen solution containing 1μg of FGF-2 was clotted in 1ml of PBS and incubated at 37°C for 24 hours. The clotted FGF-2-incorporating FS was placed in 1ml of PBS and incubated at 37°C at intervals of different times. The amount of FGF-2 in the media was quantified using standard ELISA assay. The released data of the FGF-2 was reported as the mean percentage release ± SD.

EXPERIMENT-2: FGF-2 (10ng)-incorporated FS (clotted by the method described above) was placed in 1ml of PBS and incubated at 37°C for 24 hours. Then the PBS (1ml) was transferred into a 4ml DMEM with 0.6% calf serum and Balb/c3T3 cells were cultured (Group F). After 24 and 48 hours, cells were detached by trypsinization and enumerated in a hemocytometer. The medium of control groups received PBS only (Group C).

EXPERIMENT-3: For adolescent Japanese white rabbits, 5-mm full thickness articular cartilage defects, which are not repaired spontaneously, were created in the weight-bearing area of the right femoral trochlea with a hand drill. The defects were treated in one of three ways: for Group F, the defect was filled with FS containing 7.2ng FGF-2; for Group c, the defect was filled with FS only; and for Group C, the defect was left untreated. All animals were sacrificed at 8 weeks postoperatively. For semi-quantitative analysis of the reparative tissue, sections were scored according to the histological grading scale of Pineda et al. (1992), with some modifications.

RESULTS:
EXPERIMENT-1: In the first 24 and 72 hours, 50.1 ± 5.0% and 72.0 ± 7.0% respectively, of the incorporated FGF-2 was released from the FS into the overlying media (Fig. 1).

EXPERIMENT-2: After 24 and 72 hours of incubation the cell numbers of Group F increased 1.5 ± 0.1 and 1.9 ± 0.2 fold, respectively, compared with that of Group C (Fig. 2).

EXPERIMENT-3: In Group C and f, chondrogenesis did not occur (scores: 5.2 ± 2.1, and 4.6 ± 1.3, respectively). On the other hand, the regeneration of surface articular cartilage concomitantly with the repair of subchondral bone was observed in Group F (11.0 ± 2.3) (Fig. 3).

DISCUSSION
In the previous study, we demonstrated that the regeneration of articular cartilage was stimulated by the treatment of 5-mm defects with FGF-2 because of the promotion of proliferation and migration in mesenchymal cells. The regenerative capacity of defects was significantly dependent on the incidence of replicating undifferentiated mesenchymal cells in the reparative tissue filling the defect cavities. We demonstrated that FGF-2-incorporating FS released FGF-2 in the bioactive form, and the released FGF-2 was sufficient for the induction of a chondrogenic repair response in 5-mm-diameter full-thickness defects of articular cartilage in rabbits, although fibrin sealant alone did not support articular cartilage regeneration as well as previous reports. Clinically, fibrin sealant is maneuverable and flexible enough to fill a cartilage defect so it should be a good biodegradable distributor-carrier for FGF-2.

REFERENCES

**Department of Molecular Interaction and Tissue Engineering, Institute for Frontier Medical Sciences, Kyoto University, Kyoto, Japan.

Fig.1 The time course of FGF-2 release from FGF-2-incorporated FS.
The data is expressed as the mean ± SD of the percentage of FGF-2 in the media. N=6 for each observation.

Fig.2 Growth Curves of the 3T3 cell line. On day 2 (arrow) FGF-2 released from FGF-2-incorporated FS was added to the cultures. Triplicate flasks were trypsinized and the cells were counted. The standard deviation of the different determinations did not exceed 10% of the mean. *P<0.05 compared to values obtained from control cultures.

Fig.3 Histological appearance of experiment-3 at 8 week s after the creation of the defects. (Safranin-O staining: 25×)