Introduction: There have been few studies on histological changes after Radiofrequency energy (RFE) treatment of the meniscus. RFE treatment could denature the collagen fiber and fuse the meniscal tear by heating (1). However, RFE treatment might carry a risk of thermal necrosis in meniscal tissue as well as in articular cartilage. The cell death following RFE may decrease to synthesize extracellular matrix, and surrounding matrix cannot be maintained. Therefore, it is necessary to occur the cell repopulation in RFE treated area for meniscal repair using RFE.

Autocrine motility factor (AMF) has been identified as a specific motility modifier and stimulates cell motility via binding to its receptor, 78kDa glycoprotein (2). The stimulated motility of hypoxic cells is closely related to the AMF expression (3). We thus hypothesized that AMF may be involved in enhancement of cell motility in meniscal fibrochondrocyte, following RFE treatment.

The purpose of this study was to perform meniscal repair for the avascular zone tear using RFE in animal study, and to investigate histological changes in the meniscus postoperatively. Furthermore, the expression of AMF in meniscal fibrochondrocytes was examined to analysis the mechanism of cell repopulation in the RFE-treated area.

Materials and Methods: Experimental Protocol: In this study, 20 Japanese white rabbits of female were used. Under general anesthesia, the bilateral knee joints were incised through the medial parapatellar approach. A vertical incision (about 5mm long) along the longitudinal axis of the medial meniscus was made in the avascular area of the anterior half of the meniscus. On the right knees, RFE was applied to meniscal tear using the Vulcan EAS coupled with a TAC-C probe (Smith & Nephew Endoscopy, Menlo Park, CA) under pooling of saline solution in the knee joint. The generator setting for monopolar RFE is 40W and the temperature setting is 60 Celsius. Four rabbits were killed at 0, 1, 2, 4 and 12 weeks after surgery. Following macroscopic examination of the meniscus appearance and tear stability, both medial menisci were carefully resected.

Histological and Immunohistochemical Study: The menisci were fixed in 10% formalin, dehydrated in ethanol, and embedded in paraffin. Four-micrometer cross-sections through the lesion were made perpendicular to the longitudinal axis of the meniscus. Slides were stained with hematoxylin and eosin according to standard laboratory protocols. The microscopic findings examined healing of the meniscus and meniscal fibrochondrocyte morphology. Immunohistochemical staining for AMF was based the avidin-biotin-peroxidase complex method (Vector, Burlingame, Calif., USA), using the anti-AMF polyclonal antibody. Negative control sections were treated with nonimmunized rabbit IgG as the primary antibody. Positive ratio of immunostaining for AMF expressed immunostain positive cells / immunostain positive or negative cells was calculated in 4 randomly selected high-power fields from the parameniscal synovium to the longitudinal tear.

Results: Histological examination at the baseline demonstrated fusion of collagen fiber in the RFE-treated area. Pyknosis of fibrochondrocyte nuclei was also found. One week after surgery, fusion of collagen fibers in the lacerated area was maintained in all specimens. The specimens showed an acellular area as a result of fibrochondrocyte death and pyknosis of fibrochondrocyte nuclei in the femoral surface of the RFE-treated area. Furthermore, inflammatory cell ingrowth mainly consisted of lymphocytes and histiocytes extending from parameniscal synovium were observed on the surface of the RFE-treated area. Two weeks, the acellular area expanded deeper, and both viable cells and necrotic cell with empty lacunae coexisted in the deep area of the meniscus. Fibroblast proliferation was found in the area with inflammatory cell ingrowth on the femoral surface. Four weeks after surgery, cell proliferation was diminished and fibroblasts were aligned. Twelve weeks, the size of the acellular area was reduced, and it was only seen adjacent to the femoral surface. The presence of normal fibrochondrocyte was noted in the deep area of the meniscus. Fibroblasts aligned on the surface became atrophic, and invaded into meniscus tissue from the meniscal surface. In contrast, there was no cell proliferation, no damage to fibrochondrocytes and no healing of the tear in the control group at any time points.

Positive ratio of immunostaining calculated from immunohistochemical finding using anti-AMF polyclonal antibody was 59.8% in the RFE group and 63.8% in the control group at the baseline, showing no significant difference between the two groups. However, this ratio in RFE group was significantly higher than that in the control group with time.

Discussion: This study demonstrated that RFE treatment fused the meniscal tear in rabbits just after surgery. Postoperatively, acellular area as a result of meniscal fibrochondrocyte death was found in the femoral surface of the RFE-treated meniscus, expanded deeper until 4 weeks after surgery, and had almost disappeared at 12 weeks after surgery. One week after surgery, there was inflammatory cell ingrowth mainly consisting of lymphocytes and histiocytes on the surface of RFE treated-area, and fibroblast proliferation was found 2 weeks after surgery. Finally, at 12 weeks after surgery, fibroblasts extended from the parameniscal synovium to the tear on the femoral surface, and invaded into the acellular area.

Regarding cellular repopulation of RFE-treated meniscus in rabbits, our study showed that fibroblasts extended from the parameniscal synovium on the femoral surface, and invaded into the acellular area. This result suggested that the fibroblasts derived from the synovium might participate to repopulation into the RFE-treated meniscus. In the control group, there was no increase in the positive ratio of AMF in the meniscal fibrochondrocytes at any period. In contrast, in the RFE group, there was a significant increase in the positive ratio from 1 to 12 weeks after surgery. Particularly, the positive ratio at 2 and 4 weeks markedly increased. AMF is reported to be induced by hypoxia (4), and normally expressed in chondrocytes normally (3). These results suggest that the expression of AMF induced by RFE treatment, an inducer of hypoxic atmosphere may stimulate the motility of fibrochondrocytes, and the acellular area could be repaired by the migration of the fibrochondrocytes in the body of the meniscus. Furthermore, it suggests that the cell repopulation in this process is maximally activated from 2 to 4 weeks after surgery.