INTRODUCTION:
The hamstring tendon autograft is widely used for anterior cruciate ligament reconstruction because of its superior tensile strength and lower donor site morbidity. The successful reconstruction surgery depends on not only initial graft fixation but also biologic integration of the graft. The healing process of transplanted tendon graft to bone via the development of fibrovascular tissue has been revealed in both animals and humans. In recent study, it has been demonstrated that some growth factors may promote maturity of the fibrous interface or remodeling of the bone tunnel, but the mechanism remains unclear.

To identify how the growth factors regulate the healing process between the tendon graft and bone, we assessed the expressions of basic fibroblast growth factor (bFGF), a potent mitogenic agent on a variety of cells of mesenchymal origin, and vascular endothelial growth factor (VEGF), an intensely angiogenic factor that targets vascular endothelial cells, in the interface tissue by immunohistochemical procedure.

MATERIALS AND METHODS:
Animal model: Twenty-four mature female Japanese White Rabbits (3.0-3.5 kg) were used for this study. Treatment of each animal was conducted in accordance with the guidelines for animal experimentation established at our institute. The long digital extensor tendons from bilateral hind-limbs were detached from their insertion on the lateral femoral condyle. After a 2.5 mm-diameter drill-hole was made through the proximal tibial metaphysis beginning at its lateral cortex and exiting into the joint, the tendon was pulled into the joint and was fixed through the lateral femoral condyle with the endobutton device (Fig. 1). The articular entrances of drill-holes in the tibia were placed adjacent to the original insertion site of the anterior cruciate ligaments. None of the animals were immobilized postoperatively, and each rabbit was allowed unrestricted daily activities in its own cage. Six animals each were sacrificed at one, three, six and twelve weeks after the surgery. In each animal, both hind-limbs were used for histological and immunohistochemical evaluation of the bone-tendon interface.

Histological and immunohistochemical analysis: The tibia-tendon complexes were harvested, fixed with 10% buffered formalin solution, and decalcified in 10% EDTA for 5 weeks. Each specimen was sectioned parallel to the longitudinal axis of the bone tunnel to examine the sagittal plane of the bone-tendon interface. The specimens were dehydrated through a graded ethanol series, embedded in paraffin, and cut into 4 µm sections. These sections were stained with hematoxylin and eosin, and Masson’s trichrome. Subsequent to the routine histological examination, immunohistochemical analysis was performed to evaluate the expression of some growth factors related to bone-tendon healing. Immunohistochemistry was performed by standard immunoperoxidase techniques. For immunohistochemical evaluation, antibodies to bFGF (Wako Junyaku, Osaka, Japan) and VEGF (IBL, Fujikawa, Japan) were used.

RESULTS:
At one week after surgery, the interface tissue between the tendon graft and bone was separated by a gap covered with a layer of fibroblasts. The interface was composed of loosely organized connective tissue and oval fibroblasts which showed irregular arrangement. At three weeks, the gap was disappeared and filled with the connective tissue including increased fibroblasts. At the loose part of the connective tissue in the interface, occasional vascularization was observed. At six weeks, fibroblasts were decreased as compared with the specimens at three weeks but they were integrated in their direction along the grafted tendon. Collagen fibers anchoring into the woven bone layer of the bone tunnel progressed toward the tendon. Growing vascularization, observed at three weeks, had decreased at this period. In the specimens after six and twelve weeks, fibroblasts in the interface tissue had no immunoreactivity for bFGF and VEGF antibodies.

DISCUSSION:
This study demonstrated that bFGF and VEGF may enhance the maturation of the interface tissue with vascularization. Administration of these growth factors at early phase of tendon-bone healing may accelerate the maturation. The findings in this study provide the foundation how to improve the tendon-bone healing using growth factors.

REFERENCES: