Introduction: The diameter of the bone tunnels for anterior cruciate ligament (ACL) reconstruction is occasionally drilled to a greater degree than that of the autograft. Specifically for the flexor tendon graft, orthopaedic surgeons have been apprehensive that the graft-tunnel diameter disparity may present adverse effects on graft healing within the bone tunnel. Few experimental studies, however, have been conducted to quantitatively document the effects of the disparity in ACL reconstruction. The purpose of this study using a canine model is to quantitatively assess and histologically confirm the graft-tunnel diameter disparity on healing of the doubled flexor tendon graft within the bone tunnel in ACL reconstruction.

Materials and Methods: Twenty-eight adult beagle dogs weighing 10.9 ± 0.6 kg (Mean ± SD) were divided into two groups, Groups T and L, of 14 animals each. In each group, the left ACL was resected through the medial parapatellar approach, and anatomical ACL reconstruction was performed using the doubled flexor tendon graft having a diameter of 4 mm. In Group L, a bone tunnel having a diameter of 4 mm was drilled in the tibia, so that the graft fit tightly within the bone tunnel. In Group L, a bone tunnel having a diameter of 6 mm was drilled in the tibia, so that the graft fit loosely within the bone tunnel (Fig. 1). To create a substitute, the flexor digitorum superficialis tendon having a length of 10 cm was harvested from the ipsilateral hind-limb. The tendon was sharply trimmed parallel to the fiber orientation so that the doubled tendon could be passed through a 4-mm-diameter sheath. Two #1-Ticron sutures were firmly attached at each end of the doubled tendon. For each graft, the distal end having a length of 15 mm was placed in the tibial bone tunnel, and the proximal end was routed through the over-the-top of the femoral bone. We tethered each end of the graft with the sutures to a screw inserted to the bone, applying a tension of 1 N. No animals were immobilized postoperatively, and all were allowed unrestricted daily activities in their cages. In each group, 7 animals were sacrificed at 3 and 6 weeks, respectively. At each period, 5 and 2 dogs were used for biomechanical and histological examinations, respectively. For biomechanical evaluation, the femur-graft-tibia complex was mounted on the tensile tester, so that the tunnel was positioned to allow tensile loading aligned with the long axis of the bone tunnel. Pull-out tests of the graft-tibia complex were carried out at a crosshead speed of 20 mm/min, after the sutures tethering the graft to the tibia were cut to determine anchoring strength. Histologically, the following three sites within the tibial tunnel were evaluated with light and polarized light microscopy: 1) the anterior aspect of the tendon-bone interface, 2) the posterior aspect of the tendon-bone interface, and 3) the tendon substance. Statistical comparison was made using the two-way ANOVA.

Results: 1) Histological observations: In each group, new bone formation with many osteoblasts appeared at the wall of the bone tunnel at 3 weeks. The thickness of the newly formed bone was greater in Group L than in Group T at 6 weeks. The space between the tendon and the bone (tendon-bone gap) was greater at the anterior interface than at the posterior interface in each group. At each interface, the tendon-bone gap was greater in Group L than in Group T at each period. In each group, the gap was filled with granulation tissue rich in fibroblasts and vessels at 3 weeks. In the granulation tissue observed at 3 weeks, the parallel collagen fibers connecting the graft to the bone appeared in wider areas around the graft in Group L than in Group T (Fig. 2-a, b). At 6 weeks, the perpendicular collagen fibers further increased and became more dense in Group L than in Group T. In the tendon substance within the tunnel, a number of fibroblasts with a round nucleus appeared in the interfascicular space, which was wider in Group L than in Group T, at 3 weeks, although few cells were observed in the tendon substance located in the articular space. At 6 weeks, the number of fibroblasts decreased and the nucleus appeared to be rod-shaped only in Group L.

2) Mechanical tests: At 3 weeks, all grafts in each group were pulled out from the tunnel. At 6 weeks, 2 and 3 of 5 specimens failed in the tendon substance in Groups T and L, respectively, while the remaining specimens were pulled out from the tunnel. The ultimate load of the graft-tibia complex significantly increased at 6 weeks in each group, compared to the specimens examined at 3 weeks. It was noted that there were no significant differences in the ultimate load between Groups T and L at each period (Fig. 3).

Discussion: The histological observations in this study demonstrated that the graft-tunnel diameter disparity affects healing of the flexor tendon graft within the bone tunnel. The perpendicular collagen fibers connecting the graft to the bone were generated to a greater degree in the loosely fit group than in the tightly fit group. In addition, new bone formation from the tunnel wall was further progressed in the loosely fit group than in the tightly fit group. This result implied that activities of fibroblasts and osteoblasts are affected by the degree of the graft-tunnel diameter disparity. Mechanically, however, the graft-tunnel diameter disparity of 2 mm (67 %) did not significantly affect the pull-out strength of the graft. This result may be explained by the increase of the perpendicular collagen fibers in the loosely fit group. As to the clinical relevance, graft-tunnel diameter disparity within 2 mm may be acceptable in ACL reconstruction using the flexor tendon graft.

The authors have not received anything of value from a commercial or other party related directly or indirectly to the subject of my presentation.