INTRODUCTION
The successful outcome of anterior cruciate ligament reconstruction using a soft tissue graft mainly depends on the biological anchoring of the transplanted tendon graft to the bone tunnel. The integration process, consisting of the woven bone around the graft and newly formed collagen fibers in the interface, was histologically demonstrated in several experimental models (1). Since the weakest portion shifts from the tendon-bone interface to the mid-substance of the tendon graft at 4-6 weeks after the transplantation, the biomechanical properties of the tendon-bone interface have been evaluated only in the early phase of the integration process. Some authors pointed out that the actual interface strength was higher than the measured strength value when the tendon ruptured (2). To clarify the actual interface strength throughout the entire integration process, we developed a new animal model using the tendon graft reinforced with a suture material. The purpose of this study was to demonstrate the failure behavior of the augmented tendon graft transplanted into the bone tunnel until the complete integration of the graft to the bone tunnel.

MATERIALS AND METHODS
Fifty skeletally mature female Japanese White Rabbits (3.5-4.0Kg) were used for this study. This study was carried out in accordance with the Guidelines for Animal Experimentation, Hirosaki University. The extensor digitorum longus (EDL) was detached from the femoral insertion. A No.2 Ethibond suture (Ethicon, Inc., Somerville, New Jersey, USA) was penetrated into the mid-substance of the EDL, and the muscle-tendon junction and the free end of the EDL were lock-sutured. The embedded suture was certainly existent in the tendon. A bone tunnel 2.5mm in diameter was drilled on the proximal tibia perpendicular to the long axis of the tibia and the length of the bone tunnel was measured. The graft was drawn through the bone tunnel, and was tightly fixed at the medial aspect of the tibia with the button (suture group) (Fig. 1). The contralateral limb underwent a same operation without the penetrated suture (control group). After surgery, all animals were allowed unrestricted in their cages without immobilization. Ten animals each were killed at 4, 6, 8, 12 and 16 weeks after surgery (8 for biomechanical testing and 2 for histological evaluation). For biomechanical testing, the specimen was mounted on the materials testing machine (Instron 4465; Instron Corp., Canton, Massachusetts, USA) with a specially designed clamp to allow tensile loading along the long axis of the bone tunnel. After the removal of the fixation button, the ultimate failure load was measured at a crosshead speed of 100 mm/min. Because the length of the bone tunnel varied between specimens, the failure load-to-tunnel length ratio (FTR; N/mm) was determined by normalizing the failure load by the tunnel length. A paired t-test was used to compare the biomechanical data between the two groups at each time point. One-way analysis of variance with the Scheffé test for post-hoc test was used to compare the biomechanical data at different time points within each group. The level of significance was set at p<0.05. The specimens were prepared to histologically evaluate the tendon-bone interface using hematoxylin and eosin, and Masson’s trichrome staining.

RESULTS

Biomechanical testing
In the suture group, at 4, 6 and 8 weeks, all tendon grafts were pulled out of the bone tunnel. At 12 weeks, failure mode was mixed with the pull out and the partial pull out. At 16 weeks, all tendon grafts ruptured at the mid-substance. In the control group, all tendon grafts were pulled out at 4 and 6 weeks, while ruptured at the mid-substance at 8, 12 and 16 weeks. The FTR was significantly larger in the suture group compared to the control group at 8, 12 and 16 weeks. In the suture group, significant increases of the FTR were observed at 8 and 16 weeks compared to 4 weeks, and at 12 weeks compared to 4 and 6 weeks. In the control group, there were significant increases of the FTR at 12 weeks compared to 4 and 16 weeks (Fig. 2).

Histological findings
At 4 weeks, the tendon-bone interface was filled with fibrovascular tissue. At 6 weeks, collagen fibers anchoring into the woven bone layer of the bone tunnel progressed toward the tendon graft. At 8 weeks, the nearly complete integration of the tendon graft to the bone tunnel was observed. At 12 weeks, the graft contacted the bone directly. At 16 weeks, the bone layer was well organized at the entrance of the bone tunnel. The foreign body reaction to the suture material was not observed.

DISCUSSION
In previous studies, the mid-substance tear of the tendon graft occurred even before the complete integration of the tendon-bone interface. In this biomechanical testing, the failure mode in the control group was the same manner described above. In the suture group, however, the augmented tendon graft pulled out of the bone tunnel at 8 and 12 weeks after surgery in spite of the matured tendon-bone interface observed in the histological evaluation. This suggested that the suture material contributed to prevent the mid-substance tear at 8 and 12 weeks. Furthermore, the FTR for the suture group that showed the pull out from the bone tunnel was significantly larger than that for the control group that showed the mid-substance tear at 8 and 12 weeks. This experimental model may allow to evaluate the effect of the biological treatment to accelerate the integration of the tendon-bone interface, such as gene therapy and administration of a growth factor.

REFERENCES

Fig. 1. Schematic illustration of grafting procedure.

Fig. 2. The failure load-to-tunnel length ratio of both groups.