The Process of Tendon Regeneration in an Achilles Tendon Resection Rat Model: a Macroscopic, Histological, and Biomechanical study

INTRODUCTION
Regeneration of the medial hamstrings after harvesting for anterior cruciate ligament reconstruction has been reported in several studies. However, the process of tendon regeneration has not been well described. The purpose of this study was to clarify the mechanism of tendon regeneration by investigating the process of tendon regeneration macroscopically, histologically, and biomechanically.

MATERIALS AND METHODS
Surgical procedure Fifty, adult female, Sprague-Dawley rats (Japan SLC, Shizuoka, Japan), weighing 200 to 250 g, were used. After anesthesia, the Achilles tendon in the left hind limb was removed totally using the tendon-stripping device.

Macroscopic and histological examination Rats were sacrificed at 2, 7, 30, 90, and 180 days after surgery, and the regenerate tendons were dissected. Contralateral Achilles tendons were used as a normal controls. Gross anatomic changes and microscopic remodeling were observed.

Immunohistochemistry Immunohistochemical analysis of regenerate tendons were performed using rabbit anti-transforming growth factor-β1 (TGF-β1) antibody (1:100, Santa Cruz, Delaware, CA, USA), mouse anti-type I collagen antibody (1:1000, Sigma, St Louis, MO, USA), and mouse anti-type III collagen antibody (1:1000, Sigma)

Biomechanical examination Biomechanical examination was performed on postoperative days 2, 7, 30, 90, and 180 (n = 5 for each group at each time point). Fresh regenerate tendons were dissected and stored at -20°C until testing. An AGS-G materials testing machine (Shimazu, Japan) was used to measure the biomechanical properties of each specimen. Ultimate tensile strength and stiffness were measured and compared between each group.

RESULTS
Macroscopic and histological examination The regenerate tendons formed in all specimens. In the early phase, hematoma and soft granulation tissue were uniformly observed at the harvest defect. These gradually matured with time (Figure 1), and the microscopic structure became quite similar to normal 180 days after surgery (Figure 2).

Immunohistochemistry TGF-β1 was well co-localized with inflammatory cells and fibroblasts in the regenerate tendon. The type I/type III collagen ratio in the regenerate tendon was significantly decreased in the early phase (p<0.05), but gradually increased with time (Figure 3).

Biomechanical examination Ultimate tensile strength and stiffness were significantly increased with time, though it was significantly lower at day 180 than that of normal tendon (p<0.01) (B).

DISCUSSION
The results of this study clarified the process of tendon regeneration macroscopically and histologically, which was associated with histological findings. The new tissue might increase its mechanical properties over time, and the alteration of the type I/type III collagen ratio might be the key factor for biomechanical maturation of regenerate tendon. However, the mechanical properties of the regenerate tendon were significantly inferior to those of normal tendon, even 180 days after surgery, though the histological findings were quite similar to those of normal tendon. We would still be cautious about using reharvested hamstring tendons for revision ACL surgery.

REFERENCES

Figure1: Normal Achilles tendons were very thin but rubbery (A). There were no obvious structures at the harvest defect at day 2 (B). Elastic soft granulation tissues were uniformly observed at the harvest defect at day 7 (C) and matured with time and became gradually similar to normal tendon (D-F). (Bar=100 µm)

Figure2: Normal tendon, regularly arranged collagen fiber and few spindle-shaped fibrocyte nuclei were observed(A). Many small, round-shaped cells and some spindle-shaped cells were observed(B). These cells are markedly increased, and the density of collagen fibers is also increased and the direction has become organized (C-F). (Bar=100 µm)

Figure3: The staining intensity of TGF-β1 is significantly increased only at day 7 compared with that of normal tendon (p<0.01) (A). The ratio of type I/type III collagen in the regenerate tendon is significantly decreased from day 2 to day 90 compared with normal tendon (p<0.05). It reaches its lowest level at day 7 and then gradually increases (B).

Figure 4: The ultimate tensile strength increased significantly with time, though it was significantly lower at day 180 than that of normal tendon (p<0.01) (A) Stiffness also increased significantly with time, though it was significantly lower at day 180 than that of normal tendon (p<0.01) (B)