INTRODUCTION
Previous studies have shown that, in ligament reconstruction, the tendon graft is necrotized, and then, fibroblast infiltration subsequently occurs with mechanical deterioration of the grafted tendon matrix [1, 2, 3]. No studies, however, clarified whether fibroblast infiltration deteriorates mechanical properties of the tendon matrix. This question is important to understand the mechanism of the detrimental effect of fibroblast infiltration on the grafted tendon. In addition, Yamamoto et al [4] reported that the mechanical properties of the tendon tissue are different between collagen fascicles and tendinous bundles harvested from the same tendon. Therefore, the effect of fibroblast infiltration on the acellular tendon tissue may be different between the fascicle and the bundle even in the same tendon. The purpose of this study is to clarify whether ex vivo infiltration of fibroblasts into the acellular tendon deteriorates the mechanical properties of the collagen fascicle and the tendinous bundles harvested from the same tendon.

METHODS
A total of 36 skeletally mature female Japanese White rabbits were used in this study. From 12 animals, fibroblasts in the patellar tendon (PT) were isolated and cultured using an explants culture technique.

Fibroblast infiltration into the PT ex vivo: PTs harvested from the other 24 rabbits were immersed in liquid nitrogen for 60 seconds and then thawed in saline solution at 37 degrees Celsius. This treatment was repeated 3 times. Cultured fibroblasts incubated in collagen gel were seeded onto the acellular PT at initial density of 5 x 10^6 cells/ml (the experimental group). As a control, collagen gel without cells was applied onto the PT (the control group). For each group, six specimens were examined at 3 and 6 weeks, respectively. To evaluate cellular infiltration into the PT, fibroblasts were labeled with a fluorescent dye (Propidium Iodide). We determined the total number of cells scattering in a unit volume of the PT using a confocal laser microscope (CLM).

Mechanical evaluation: In each specimen, collagen fascicles having an approximately 300-micrometer diameter and a 15-mm length were carefully dissected in parallel to the long axis (Fig. 1). A specially designed micro-tensile tester was used to determine the mechanical properties of the fascicle [4]. We also evaluated the mechanical properties of the tendinous bundle, harvested from the same specimen, having an approximately 4-mm width, a 2-mm thickness, and a 20-mm length. Tensile testing was performed using a conventional tensile tester. For each mechanical evaluation, the strain at the tendon substance was measured with a video dimension analyzer. A two-way ANOVA was performed for statistical analysis.

RESULTS
In the experimental group, a number of fibroblasts were found in the surface portion of the PT at 3 weeks after seeding. In the core portion, fibroblasts were observed at 6 weeks (Fig. 2). Total numbers of cells scattering in a unit volume of the PTs were 5460 +/- 290 and 13600 +/- 5790 at 3 and 6 weeks, respectively. Concerning the mechanical properties of the fascicle, the elastic modulus significantly decreased with time in both the groups (Fig. 3-a). The modulus values measured at 3 weeks were 41% and 94% of the normal PT in the experimental and control groups, respectively, while those measured at 6 weeks were 30% and 60%, respectively. The elastic modulus of the fascicle was significantly lower in the experimental group than in the control group. On the other hand, we did not find significant differences in the elastic modulus of the bundle between the experimental and control groups (Fig. 3-b). Even in the control group, the elastic modulus of the bundle was drastically reduced to 32% and 26% of the normal PT at 3 and 6 weeks, respectively.

DISCUSSION
In this study, we have developed a unique technique in which fibroblast infiltration into the acellular tendon tissue is induced ex vivo. Based on it, this study demonstrated that ex vivo infiltration of fibroblasts into the acellular tendon deteriorates the mechanical properties of the collagen fascicle harvested from the tendon. It is considered that this is an essential effect of fibroblast infiltration on the unit composed of the tendon. However, we did not find significant effects of fibroblast infiltration on the mechanical properties of the thick bundle harvested from the same specimens. In this study, tendon specimens were incubated without any stress. Therefore, the drastic reduction of the elastic modulus in the bundle was considered to be caused by stress-deprivation. This striking effect may mask the effect of fibroblast infiltration on each fascicle in the bundle.

REFERENCES

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