INTRODUCTION:
Autologous tissue grafts have been used for irreparable massive rotator cuff tears. Successful rotator cuff reconstruction with an autologous tissue graft requires the solid integration of the graft at the bone-tendon interface and the tendon-tendon interface. In order to develop appropriate strategies to enhance tendon healing, it is necessary to clarify the fate of the graft and host cells. We used a transplantation model with green fluorescent protein (GFP) transgenic rats and wild-type rats to simulate autologous implantation. GFP has no immunological rejection in vivo and thus, this implantation model theoretically simulates autologous implantation. The purpose of this study was to examine the behavior of the host and graft cells in the early remodeling process after tendon implantation by using GFP transgenic rats.

MATERIALS AND METHODS:
Twelve-week-old female GFP transgenic rats (n = 20) and Sprague Dawley (SD) wild-type rats (n = 20) were used; these were genetically identical, except for the GFP transgene. These rats were anesthetized with intraperitoneal injections of sodium pentobarbital. A 3-cm incision was made over the shoulder joint. Tendinous defects, 5-mm long and involving the full width of the tendon, were introduced close to the supraspinatus tendon insertion (Fig. 1). The remaining distal fibrous stump was resected to expose the greater tuberosity. In Group A, Achilles tendons of wild-type rats were implanted in the defect of the GFP rats. Two drill holes were created by drilling from the lateral aspect of the humerus to the supraspinatus tendon insertion. The distal end of the tendon was sutured with 5-0 nylon, each suture passing through a drill hole. In Group B, Achilles tendons of the GFP rats were implanted in the defect of wild-type rats in the same manner as in Group A. The treated rats (n = 4) were sacrificed immediately after transplantation (time zero) and at 1, 3, 7, and 28 days after implantation. Their shoulders, including the deltoid muscle, the rotator cuff muscles, the proximal one-third of the humerus, and the glenoid were harvested and cut into 14-μm serial sagittal frozen sections. These sections were stained with hematoxylin and cosin (HE) and observed with a confocal laser-scanning microscope (LSM 510, Zeiss) to examine the survival of the GFP-positive cells. The area of interest was divided into 4 different zones, the proximal, bursal, articular, and distal sides. We counted the GFP-positive cells and performed nuclei staining using propidium iodide to determine the cell number in a randomly selected area at a magnification of ×200 (0.2 × 0.2 mm). Subsequently, the proportion of GFP-positive cells was assessed at each time period.

RESULTS:
Examination with HE staining at 1 day after implantation revealed that the bone-tendon interface was composed of fibrous tissue and that the grafted tendon was surrounded by inflammatory cells. Spindle-shaped cells (fibroblast-like cells) appeared in the grafted tendon at 7 days, and these cells filled the grafted tendon to its full extent at 28 days. In addition, the vascularization increased in 7 days. Lymphocyte invasion of the graft, indicating immunological rejection by the host, was not found in any sections. In the sections from Group A at time zero, host cells demonstrated GFP signals under the fluorescent microscope, whereas the graft showed no signals. At 1 day after implantation, in Group A, a few oval- to spindle-shaped GFP-positive cells were found on the bursal, proximal, and articular sides of the grafted tendon (Fig. 2A, B). At 7 days, in Group A, the number of GFP-positive cells increased on the bursal, proximal, and articular sides (Fig. 3A). In Group B, the GFP-positive cells remained on the proximal side of the grafted tendon (Fig. 4B). There were no GFP cells in the distal portion (Fig. 4A). At 28 days, in Group A, a large number of GFP-positive host cells were found in each portion of the graft (Fig. 3B), while in Group B, GFP-positive graft cells were not found anywhere.

DISCUSSION AND CONCLUSION:
Several studies showed that the progenitor cells from the bone of the tendon insertion and the subacromial bursa contributed to the repair process of the rotator cuff tear, and these cells started proliferating at several weeks after autologous tendon implantation. However, whether the host or graft cells contribute to tendon repair and the localization of both these cells during tendon repair has not been clarified. Our study showed that the proliferation of the host cells in the grafted tendon started at 1 day after implantation. Host cell invasion occurred mainly from the subacromial bursa, the proximal tendon, and the bone of the tendon insertion. Additionally, GFP-positive cells invaded the grafted tendon through the articular side. The number of graft cells decreased with time. Our results demonstrated the fate of host and graft cells during the early repair process after tendon implantation for massive rotator cuff tear.