BEHAVIOR AND PROLIFERATION OF BONE MARROW AND GRAFT DERIVED CELLS IN BONE TUNNEL AFTER SIMULATED AUTOLOGOUS TENDON GRAFT

Introduction:
Autologous tendon graft into the bone tunnel has widely been used for anterior cruciate ligament (ACL) reconstruction, while biomechanical and biological properties of reconstructed ACL do not return to those of normal ACL (1). Integration of grafted tendon at the bone-tendon interface and the remodeling of grafted tendon are necessary for the recovery of normal properties of ACL (2, 3). Healing process after transplantation involves the formation of fibrous tissue at the bone-tendon interface in early phase and necrosis of graft followed by incorporation of cells, in which bone marrow derived (host) and graft derived (graft) cells participate (4). On the 51st ORS Annual Meeting, we developed a simulated autologous transplantation model between green fluorescent protein (GFP) transgenic rats and wild-type rats. We demonstrated survival of host and graft cells after tendon grafting and demonstrated that host mesenchymal cells rather than graft cells contributed to the repair of the bone-tendon interface and the remodeling of the graft after autologous tendon grafting (5). However, the function of the host and graft cells during the early healing process after transplantation has not been elucidated. Understanding the cellular function as well as survival of both host and graft cells after autologous tendon transplantation should be the pilot data to engineer the healing process for regenerating the normal ACL properties. The objective of this study was to examine the fate and proliferation of the host and graft cells during the early healing process after autologous transplantation of tendon grafts at the bone-tendon interface and grafted tendon with the GFP rat model.

Materials and Methods:
Twelve-week-old female green fluorescent protein (GFP) transgenic rats (n=8) and SD wild-type rats (n=8), which were genetically identical to each other except for transgenes, were used. In Group A, Achilles tendons of wild-type rats were harvested and each tendon was transplanted into the bone tunnel of the GFP rats, which was drilled from intercondyler notch to lateral epicondyle of the femur in 2 mm diameter. End of the tendon was sutured with periosteum at the epicondyle outside the joint and the opposite side was sutured with patellar tendon to add mechanical stimuli to the tendon graft. In group B, Achilles tendon of GFP rats were transplanted into the bone tunnel of the wild-type rats in a same manner with group A (Fig. 1). At 3 and 7 days after the transplantation, bromodeoxyuridine (BrdU) (10mg/kg body wt.Sigma) was administered with intraperitontial injection to each of the rats, then distal epiphyses of femurs were harvested and sagittal serial frozen sections were cut at the thickness of 14um. In vivo BrdU immunohistochemical stain was performed to monitor the DNA replication in the host and graft cells of each of the rats. These sections were observed with a confocal laser scanning microscopy (LSM 510, Zeiss, Germany) to examine the GFP positive cells and BrdU positive cells. Serial sections were stained with hematoxylin and eosin (HE).

Results:
At 3 and 7 days after the transplantation, fibroblasts migrated into the bone-tendon interface, which was filled with the coarse fibrous tissue in both groups. Necrosis was found inside the grafted tendon, in which few fibroblasts were remained. There was no invasion of inflammatory cells in the graft to indicate the immunological rejection by the host. In Group A, at 3 days after transplantation, GFP positive cells which were derived from host bone marrow cells appeared in the grafted tendon (Fig. 2A). At 7 days after transplantation, the proportion of GFP positive cells increased (Fig. 2B). Some of these GFP positive cells showed the signal of BrdU at 3 and 7days (Fig. 4A). In Group B the proportion of GFP positive cells which were derived from the grafted tendon gradually decreased as time passed (Fig 3A,B). Some of these GFP positive cells still showed the signal of BrdU at 3 and 7 days (Fig. 4B).

Discussion:
Healing process after the autologous tendon transplantation in the bone tunnel consists of the initial integration of fibrous tissue at the bone-tendon interface, necrosis of graft, and incorporation of cells in the graft (4). We have demonstrated that graft cells disappear from graft in the early healing phase, followed by the replacement of the graft by host cells (5). In sections of HE staining, the bone-tendon interface was formed by the coarse fibrous tissue and necrosis, remaining few fibroblasts, which was found inside the graft at 3 and 7 days after the transplantation. In sections of laser scanning microscopy in Group A, only host cells, which showed GFP positive signals, were detected in both the interface and inside the graft at both 3 and 7 days. Graft cells, which showed GFP positive signals in Group B, were found in the section at 3 days. But in the section at 7 days, the proportion of GFP signal positive graft cells decreased. Regarding proliferation, there were GFP positive host cells, which were positively stained with BrdU, at 3 and 7 days in Group A. In Group B, GFP positive cells, which were derived from grafted tendon cells, also showed the positive signal of BrdU at 3 and 7 days. It was found that both host and graft cells proliferate in earlier healing phase after autologous transplantation although graft cells disappear from graft in the early healing phase, followed by the replacement of the graft by host cells. In conclusion, both host and graft cells have a potential to proliferate in earlier healing phase after autologous transplantation.

References:

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Fig 1. Groups and tunnel location.
Fig 2A: Group A at 3 days. B: Group A at 7 days.
Fig 2B. A: Group A at 3 days. B: Group B at 7 days.
Fig 3A. A: Group A at 7 days with BrdU. B: Group B at 7days with BrdU.
(Green: GFP. Red: BrdU)