ACL Reconstruction Using Autologous Tendon Graft Wrapped with Cell Sheet of ACL-derived CD34+ cells

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Introduction

We have recently reported that the ruptured and septum regions of the human ACL contain numerous vascular-derived stem cells and that these ACL-derived stem cells have potential for high expansion and multilineage differentiation (1). Moreover, we also revealed that these cells contribute to the tendon-bone healing in a rat model of ACL reconstruction via enhancement of angiogenesis and osteogenesis (2). In this previous study, the cells were injected into knee joint capsules, which may not be the best technique to employ in a clinical situation due to the low efficiency of cell transplantation and risk of side effects. In the present study, we developed the cell sheet wrapped graft and performed experiments to prove the hypothesis that ACL-derived CD34+ cell sheet transplantation as a wrapped graft is more efficient than other techniques for prompt recovery after ACL reconstruction, not only in bone-tendon healing but also in graft maturation.

Materials and Methods

Cell sheet preparation using ACL-derived cells

ACL-derived cells were obtained from human ACL rupture sites with collagenase digestion. CD34+ cells were isolated by fluorescence-activated cell sorting. Subjects signed an informed consent prior to surgery, and this protocol was approved by our University’s Institutional Review Board. Cell sheets were constructed using temperature-responsive culture plates with ACL-derived CD34+ cells (3).

ACL reconstruction and cell sheet transplantation

ACL reconstruction was performed in immunodeficient rats using autologous flexor digitorum longus tendon as a graft (4). The grafts were wrapped with a cell sheet of 5x10^6 ACL-derived CD34+ cells in the Sheet group, and the same dose of these cells in PBS were injected into the knee capsule in the Injection group.

Results

Ex vivo cell migration assay in cell sheet

Ex vivo migration assay was performed to confirm cell migration into the graft, and migrated cells were detected as Dil+ cells (Figure 1A).

ELISA for VEGF secretion from cell sheet

The ELISA study revealed higher expression of VEGF in sheet cultured cells than in normal monolayer cultured cells (Figure 1B).

Cell incorporation effect of cell sheet wrapped graft transplantation

To track the transplanted cells in vivo, the cells were stained with DiI and detected histologically 1 week after transplantation. The numbers of DiI+ cells at the bone tunnel and in the grafted tendon were significantly higher in the Sheet group than in the Injection group (Figure 2A, B).

Enhancement of graft healing

Vascular staining with Isocletin B4 demonstrated enhancement of vascularization in the grafted tendon in the Sheet group at week 2 compared with the other groups (Figure 3A). Immunohistological evaluation with anti-type III collagen (Col3) antibody demonstrated enhancement of grafted tendon healing in the Sheet group compared with the other groups (Figure 3B).

Enhancement of vascularization and osteogenesis at bone tunnel sites

Enhanced angiogenesis and osteogenesis by paracrine effect of the transplanted cells on recipients’ cells were confirmed by immunostaining for rat-specific markers. Capillary density with isocletin B4 at week 2 was significantly greater in the Sheet group than in the other groups (Figure 4A). Osteoblast (OB) density with rat specific osteocalcin antibody at week 2 was also significantly higher in the Sheet group than in the other groups (Figure 4B).

Human cell-derived vasculogenesis and osteogenesis

Differentiated human endothelial cells (ECs) and OBs were identified as human-specific CD31 positive cells and osteocalcin positive cells. The numbers of human-derived EC and OB were significantly greater in the Sheet group than in the other groups (Figure 4C, D).

Preoperative recovery and graft maturation

Immunofluorescence staining for neurofilament at week 2 showed enhancement of preoperative recovery in the Sheet group, and the number of neurofilaments was significantly greater in the Sheet group than in the other groups (Figure 5A). Also, immunofluorescence staining with smooth muscle actin (SMA) at week 2 displayed enhancement of graft maturation in the Sheet group (Figure 5B).

Histological evidence of bone-tendon healing

Histological evaluation was performed with Masson’s trichrome staining to assess the tendon-bone healing at week 2. The areas of the tendon-bone interface and collagen fibers present in the interface were measured by Scion Image analysis software. Quantitative analysis demonstrated that the total collagen fiber area was significantly greater in the Sheet group than in the other groups (Figure 6A). The area of oblique collagen fiber similar to Sharpey’s fiber at week 2 was significantly greater in the Sheet group than in the other groups (Figure 6B).

Biomechanical testing

Functional recovery of ligament injury in each group was evaluated by failure load of biomechanical tensile test at week 8. Failure load of tensile test demonstrated that biomechanical strength was significantly higher in the Sheet group than in the other groups (Figure 7).

Discussion

We demonstrate that the cell sheet wrapped graft using ACL-derived CD34+ cells provides a greater amount of cell administration not only at bone tunnel site but also around/into grafted tendon, resulting in enhancement of the healing of bone-tendon junction and grafted tendon including preoperative recovery, graft maturation, and increased biomechanical strength in rat ACL reconstruction models. This technique of ACL-derived CD34+ cell sheet wrapped graft represents a promising treatment to promote prompt recovery after ACL reconstructive surgery.

References