Regenerative Capacity of Synovium-Derived Mesenchymal Stem Cells into Rabbit Partial-Thickness Chondral Defect through Intra-articular Injection

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INTRODUCTION
Damage in articular cartilage has limited possibility of spontaneous healing, and may progress to osteoarthritis which may be continued during the rest of life time with further deterioration. Synovium-derived stem cells (SDSCs) are known to have capacities for proliferation and chondrogenic differentiation; therefore they have possibilities to be used as a cell source for cartilage cell therapy (1). The surface of degenerative cartilage are deprived of proteoglycans that inhibit cell attachment onto the chondral surface, by mechanical damage to expose fibronectin, type II, and type VI collagen which may help cell attachment (2). Previously, we demonstrated that ChABC enhanced the expression of fibronectin molecule through degradation of chondroitin sulfate in partial chondral defect. Also, augmented exposure of fibronectin by treatment of ChABC appeared to increase the adhesion of SDSCs to the chondral defect region in vivo after intra-articular injection. In the present study, therefore, we evaluated in vivo model to regenerative capacity of adhesive SDSCs in the damaged articular cartilage.

MATERIAL AND METHODS
Isolation of SDSCs and Tracking dye labeling: Synovial membrane obtained from knee joint of New Zealand white rabbits was minced in PBS solution and digested in 0.02% collagenase overnight at 37°C. Cells were plated and maintained in DMEM containing 10% FBS. Ex vivo analysis of attached SDSCs on a partial chondral defect of rabbit: Partial-thickness chondral defects (3×6 mm wide, 0.2 mm deep) were created in the thochlear groove of the femurs of adult rabbit. The defects were filled with DiI-labeled SDSC suspension, which consisted of 105 cells in 50 μl PBS, and left stationary for 5, 10, 30 and 60 minutes. Then, explants transferred to a new well and attached cells on the defect were harvested with trypsin-EDTA. The number of attached cells on the defect was measured by the MTS method. In ChABC treatment group, the defects were digested with 100 μl of chondroitinase ABC solution (0.1 U/ml). Control specimens were similarly incubated, but in 100 μl of PBS for 15 min. For function-blocking experiments, we utilized antibody that specifically recognize the fibronectin after chondroitinase ABC digestion. Surgical procedure and ChABC treatment: After general anesthesia, partial-thickness chondral lesion was made in the thochlear groove (0.2 mm deep, 3 mm wide and 6 mm long) of the knee joint of white rabbits with a custom-made surgical tool. At seventh day from surgery, the rabbit knees were injected intraarticularly with an enzyme-containing saline solution (200 μl of physiological saline containing chondroitinase ABC (ChABC) at 0.1 U/joint). Left knees were injected with physiological saline as controls. Intraarticular injection of SDSCs: At eighth day from surgery, 500 μl of DiI-labeled cell suspension containing 1×10^7 SDSCs was injected into the knee joint. Histology: The dissected samples were embedded in paraffin blocks, cut into 5 μm thickness, and subjected to H&E and corresponding fluorescence of the consecutive serial section that proved the presence of DiI-labeled SDSCs.

RESULTS
Ex vivo effects of ChABC on the adhesive capacity of SDSCs: Attachment of SDSCs on the chondral defect region was evaluated by MTS assay at different incubation time points (Fig. 1). Using this qualitative method, the number of SDSCs showed an intensively increase in the ChABC-treated group from 20 minutes. However, few cells were found on the region of defect in the saline-treated group, and there was a statistically significant difference of the number of attached SDSCs between the ChABC-treated group and the saline-treated group. Pretreatment with anti-fibronectin antibody before SDSCs injection into joint significantly reduced ChABC-induced cell attachment (Fig. 5A), suggesting the increasing attachment of SDSCs to the cartilage matrix might be in large part responsible for exposure of fibronectin. Histologically, many DiI-labeled SDSCs were shown to be on the partial-thickness cartilage defect of the femoral groove as determined by H&E and corresponding fluorescence of the consecutive serial section that proved the presence of DiI-labeled SDSCs.

DISCUSSION
We demonstrated that augmented exposure of fibronectin by ChABC appeared to increase the adhesion of SDSCs to the chondral defect region ex vivo and in vivo after intraarticular injection. The results of this study indicate that the cartilage repair can be enhanced by removal of the anti-adhesive molecules and intraarticular injection of SDSCs to the chondral defect. In addition, we showed that the attached SDSCs survived, but the repaired tissue exhibited a fibrocartilage-like appearance.

REFERENCES

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