Tendon Repair with Multilayer Acellular Tendon Slices and Bone Marrow Stromal Cells: An In Vivo Animal Study

INTRODUCTION:
We have developed a novel technique for tendon repair using native acellular tendon slices seeded with bone marrow stromal cells (BMSC). The purpose of this study is to investigate the viability and differentiation of BMSC seeded on the multilayer tendon slices in a rabbit tendon repair model in vivo.

METHODS:
BMSC Harvest and Culture: Bone marrow was aspirated from both tibiae of 15 adult rabbits and centrifuged. The supernatant was removed and the bone marrow cells were cultured in minimal essential medium with Earle’s salts, 10% fetal calf serum and 5% antibiotics. The adherent cells, by convention BMSC, were harvested in the second passage to produce a cell suspension (5.0x10⁶ cells/mL). For cell tracking, BMSC from 6 rabbits were stained with PKH26 fluorescent marker (Sigma).

Sliced Tendon Scaffold: The infraspinatus tendons were harvested from 15 dogs, which were euthanized for other studies. Tendons were immersed in liquid nitrogen and then thawed in saline solution. This procedure was repeated five times. Then the tendons were incubated in nuclease solution (RNase, 5µg/mL Roche) for 12h. The trimmed tendon (10x10mm) was fixed on a cryostat (Leica CM1850) and sliced at a thickness of 50µm. Ten slices were placed in a culture dish. The dish was immediately placed in an incubator for 10 minutes to thaw.

Composite of BMSC and Multilayer Sliced Tendon Scaffold: The BMSC solution was added to the slices and cultured for one day (Figure 1A). Just before the implantation, the tendon slices with the cells were detached carefully and bundled together. Both ends of the bundled slices were fastened with 4-0 Ethilon suture (Figure 1B).

Surgical Procedures: The rabbits were anesthetized with intravenous Ketamine (35mg/kg), acepromazine (1mg/kg) and Xylazine (5mg/kg). A longitudinal incision was made on the knee and the patellar tendon was exposed. A 10 mm x 3 mm defect (length x width) was made in the middle of the tendon. The composite of BMSC and sliced tendon scaffold was sutured into the defect (Figure 1C). The membrane of the subcutaneous bursa was harvested and sutured covering the composite (Figure 1D). In 9 rabbits (6 for PCR and 3 for histological analyses), tendon slices without BMSC were implanted in the defect of the collateral knee as a control. Two weeks after operation, the rabbits were sacrificed by the intravenous administration of a lethal dose pentobarbital and the implanted composite was excised (Figure 1E).

Cell Tracking: The fluorescent stained BMSC were examined by a laser scanning confocal microscope (LSM310, Zeiss). BMSC in the composites before the operation and in the composites excised 14 days after the operation (n=6).

Histology: Paraffin embedded 5µm thickness sections were made for histological assessment, including composites before the operation and the composites 14 days after the operation (n=3). The tendon slices without BMSC implanted in the defect were also examined 14 days after the operation (n=3).

Gene Expression: A quantitative real-time reverse transcription-polymerase chain reaction (RT-PCR) was performed to measure the gene expression levels of Tenomodulin (differentiation marker to tenocytes), Collagen type I, Collagen type III, MMP2 (gelatinase), MMP3 (stromelysin) and MMP13 (collagenase). The expression level was normalized to that of GAPDH. The composite with BMSC and the tendon slices without BMSC 14 days after the operation were evaluated (n=6).

Statistics: The results of the gene expression were analyzed by the Wilcoxon signed ranks test (p<0.05).

RESULTS:

Gene Expression: Significantly higher gene expression was detected in the composite with BMSC than in the tendon slices without BMSC for Tenomodulin (p=0.028), Collagen type III (p=0.028), MMP3 (p=0.028) and MMP13 (p=0.046). Collagen type I expression was lower in the composite with BMSC than that in the tendon slices without BMSC (p=0.046) (Figure 3).

DISCUSSION:
We have developed a novel technique to create an engineered tendon substitute using acellular tendon slices at a thickness of 50µm and seeding the slices with cultured BMSC. We showed that BMSC could survive in vivo; the increased Tenomodulin expression suggests that BMSC might express a tendon phenotype in vivo. The changes of collagen and MMP expressions suggest that the BMSC also have an effect on collagen metabolism. This new composite might be useful as a model of tendon tissue engineering.

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