Rotator Cuff Regeneration with Bone Marrow-derived Mesenchymal Stem Cells in a Rabbit Model

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INTRODUCTION
A rotator cuff rupture is considered to be difficult to repair by itself until now. Various surgical techniques, including musculotendinous transfers and patch grafts using biological or synthetic materials, have been used for the treatment of irreparable tears. However, musculotendinous transfers led to sacrifice the normal tissues, and patch grafts become mechanically weaker over time because of foreign body reactions or infection. Therefore, new methods for the treatment of irreparable rotator cuff tears must be developed. In recent years, regeneration medicine is being paid attention and the tissue engineering technique is introduced to various medical fields. We described the successful results that tendon insertion was regenerated by poly-glycolic acid (PGA) sheet in rabbit’s model. These results, however, had some weak points that it was late to regenerate the fibrocartilaginous tendon insertion, and that the regenerated tissues mainly consisted of type III collagen. Another promising method to use non-differentiate progenitor cells of musculoskeletal tissues to regenerate soft and hard tissues has utilized noncommitted progenitor cells of musculoskeletal tissues to regenerate soft and hard tissues. These cells, termed mesenchymal stem cells (MSCs), were isolated from a small volume of bone marrow aspirate and culture-expanded without undergoing differentiation to more advanced cell types. The hypotheses are that the PGA sheet scaffold with seeded MSCs could enhance type I collagen products and increase the mechanical strength of regenerated tendon in vivo.

METHOD
The PGA sheet were cut and doubled measuring 10×5×1 mm as a scaffold. Bone marrow-derived MSCs (bMSCs) were isolated from the tibia of 30 Japanese white rabbits under sterile conditions, after culturing we seeded these collected cells onto each PGA scaffold by pipetting 104-105 L aliquots of the cell suspension (1.0 × 10^5 cells). The cell-polymer complex was incubated at 37 degrees Celsius in a humidified chamber of 5% carbon dioxide overnight. The deficit of infraspinatus tendons of Japanese white rabbits were reconstructed with the PGA sheet (PGA group) (n=30), and that with cultured MSCs (MSC group) (n=30). They were sacrificed at 4, 8 and 16 weeks after operation. Continuous sections (5 μm thick) were cut in the transverse plane in the middles of tendons, and stained with hematoxylin and eosin, Safranin-O, and Azan stain for the histological characterization of tissue composition, and the histological findings were evaluated at both of tendon proper and the point of tendon insertion. Furthermore, type I and type III collagen in the reparative tissues was identified and examined immunohistochemically. In order to evaluate quantitatively, we utilized tendon maturing scoring system consisted of six histological parameters including cellularity, fibrocytes vascularity, fiber diameter, cells parallel, fibers parallel, insertion histological pattern and collagen immunohistochemical findings. They were also evaluated biomechanically by measuring ultimate mechanical strengths and Young moduli at 4 and 16 weeks by a conventional tensile tester. Statistical analyses of the tendon maturing score and the mechanical properties were performed using 1-way analyses of variance and Fisher protected least significance post hoc test; a p value of <0.05 was considered to be statistically significant.

RESULT
At 4 weeks after operation, there were no histological findings and statistically significant tendon maturing scores in these two groups. At 8 weeks, although the PGA fibers were remained yet and mild foreign body reactions were seen, fibrocartilage layer was found regularly in insertion in the PGA group. On the other hand, in the MSC group not only the fibrocartilage layer but also the Sharpey’s fibers were observed in insertion. At 16 weeks after operation, in both group we found the four layer cartilage pillar pattern and the PGA fibers were completely disappeared. In immunohistochemical staining, more type I collagen could be found than type III at 16 weeks in the MSC group while more type III collagen could be found than type I in the PGA group. The tendon maturing score had a statistically significance at 8 and 16 weeks after operation between PGA and MSC group. The results of mechanical properties show that the regenerated tendons in the MSC group had adequate ultimate tensile strengths (3.70 ± 0.27MPa) than the PGA group (2.11 ± 0.90MPa) at 16 weeks after operation. There was no statistically significance about the Young’s Moduli at 16 weeks (PGA 4.70±0.94, MSC 4.97±1.33). There was no statistically significance at 4 weeks after operation. All tendon-bone complexes failed at the point of tendon insertion.

DISCUSSION
Previous studies have shown that differentiation of bMSCs can be adopted by the specific surrounding environment. It may be reasonable to expect that the bMSCs could differentiate as well as promote the regeneration of bone and fibrocartilage-like tissue under the specific environmental conditions of the tendon-bone interface. We therefore utilized the bMSCs to the PGA sheet that had a potential of regenerating the tendon-bone insertion, and achieved better results that fibrocartilaginous tissues and Sharpey’s fibers were observed at 8 weeks after operation than those we had reported.

The main component of extracellular matrices in ligaments and tendons is type I collagen. The mechanical properties of regenerated tissues, which predominantly consist of type III collagen, are likely to be inferior to those of the native tissues. To improve the mechanical properties of regenerated tendon, it is essential to increase type I collagen content in the regenerated tissues. We had regenerated type I collagen-rich rotator cuff tendon using PGA sheets seeded bMSCs and the regenerated tendon had an adequate mechanical strength in those rabbits model.

A graft created from the patient’s own cells, obtained from a biopsy sample, expanded in vitro, and seeded onto synthetic, biocompatible, biodegradable polymer scaffolds, would avoid the potential risks and complications observed with other currently used techniques and without risk of rejection. We concluded that bMSCs had a good capacity of regenerating tendon-bone insertion and tendon proper including much type I collagen. This method is quite useful for regeneration of a rotator cuff defect in clinical application.

The present study was limited by the difficulty of replicating human disease in animal models. Our experimentally-created acute rotator-cuff defects do not reflect chronic rotator-cuff tears, in which the remaining tendon and muscle may be attenuated or atrophied and retracted in human patients. However, the rabbit rotator cuff defect models are frequently utilized in experimental studies on rotator cuff regeneration. We therefore recognized these models as being the most appropriate. Furthermore, we do not yet know the mechanism by which MSCs enhance the regeneration of tendon insertion. The fate of donor cells seeded on a scaffold after implantation into a host body has not been clarified. Several studies have demonstrated that the host cells gradually replace the implanted donor cells over time. The authors showed a decline in donor cells accompanied by an influx of host cells into the repair tissue. Finally, there is no control group of rotator cuff defect model in the present study. Our previous report demonstrated similar results to the present study, but the results in the present study do appear to be both superior and more revealing than previous results.