ENHANCEMENT OF TENDON HEALING USING BONE MARROW DERIVED MESENCHYMAL STEM CELLS IN ROTATOR CUFF TENDON TEAR OF A RABBIT MODEL

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Introduction: Rotator cuff tear of the shoulder joint is a very common cause of shoulder disability and pain. Surgical repair of the torn tendon is the most commonly performed optional treatment. In massive rotator cuff tear with 5cm sized, however, it is very difficult to repair the tendon anatomically. Several surgical tools, including tendon transfer, partial repair, hemiarthroplasty, auto/allograft and artificial tissue graft have been introduced to solve this troublesome case, but their results were very disappointing.

Mesenchymal stem cells (MSCs) are undifferentiated cells that have multilineage developmental potentials and serve as long-lasting precursors which contribute to the regeneration of mesenchymal tissues, such as bone, cartilage, tendon, and ligament. In this study, we applied the mesenchymal stem cell-seeded resorbable scaffold into the defect of rotator cuff tendon in the rabbit model. The purpose of this study is to compare the histologic feature and collagen expression pattern between the cell-seeded scaffold and cell-free scaffold on the repaired tendon. To our best knowledge, this study is the first report to investigate the tendon healing on the rotator cuff tendon with mesenchymal stem cell.

Materials and Methods:

Isolation of MSCs from rabbit bone marrow aspirates, and culture
Bone marrow (7-10 ml) was harvested from the iliac crests of 36 male New Zealand White rabbits weighing 2.0 to 2.5 kg each. The bone marrow samples were layered on to Percoll (density 1.073/ml; Gibco) and centrifuged at 2500 rpm for 20 min at 4°C. We define MSCs as the adherent cells resulting from culture of a bone marrow aspirate on a tissue culture plate. The dispersed cells were plated at a density of 1 x 10⁶ per 75-T-flask, encapsulated, cells were harvested from Petri-dish culture by treatment with 0.25% trypsin-EDTA when they reached about 80% confluence. The cells counted and their membranes were labeled with PKH-26 fluorescent dye (PKH-26 Red Fluorescent Cell Linker Kit, sigma, St. Louis, MO). This cell concentration in three-dimensions is equivalent to a plating density of 2 x 10⁵ cells/cm². The cells were then cultured in Minimum Essential Medium, Alpha Modification (Alpha MEM) with 10% fetal bovine serum (FBS) and 1% antibiotic/antimycotic in a humidified chamber with 5% CO₂ at 37°C. An OPLA-scaffold is composed of a biodegradable material.

Bone marrow cell culture in an OPLA-Scaffold and PKH-26 Labeling
For OPLA scaffold (diameter 4.2-5.2 mm ; BD biosciences, MA) encapsulation, cells were harvested from Petri-dish culture by treatment with 0.25% trypsin-EDTA when they reached about 80% confluence. The cells counted and their membranes were labeled with PKH-26 fluorescent dye (PKH-26 Red Fluorescent Cell Linker Kit, sigma, St. Louis, MO). This cell concentration in three-dimensions is equivalent to a plating density of 2 x 10⁵ cells/cm². The cells were then cultured in Minimum Essential Medium, Alpha Modification (Alpha MEM) with 10% fetal bovine serum (FBS) and 1% antibiotic/antimycotic in a humidified chamber with 5% CO₂ at 37°C. An OPLA-scaffold is composed of a biodegradable material.

Histological and Immunohistochemical analysis
The samples were harvested 2, 4 and 6 weeks after implantation. Digital images were acquired using both fluorescent microscope and fluorescence microscopy. For Histological and Immunohistochemical staining, the tissue samples were fixed in 10% formalin, dehydrated in ethanol, and then subjected to a series of organic solvent and embedded in paraffin. Sections 5 μm thick were cut and stained with hematoxylin and eosin. Immunohistochemical stainings for collagen I was performed using mouse monoclonal antibody against type-I collagen (3G2, 1:100; Serotec, UK). The expression of Immunohistochemical stainings for collagen I was higher in the scaffold with MSCs than in the scaffold without MSCs (Fig 6, 7). The expression of Immunohistochemical stainings for collagen II, however, was not different between the scaffold with MSCs and the scaffold without MSCs (Fig 8, 9).

Discussion & Conclusion
This study demonstrated that many MSCs in the scaffold could survive after implantation in the rabbit rotator cuff defect. Furthermore, the generation of type I collagen increased more in the scaffold with MSCs than that of scaffold alone. It was thought that MSCs promoted the tendon healing by producing collagen type I when they were applied at the tendon defect. We believe this study would serve as the important step toward the cell therapy of the rotator cuff tendon tear in the shoulder joint.

References