ENHANCEMENT OF TENDON HEALING USING BONE MARROW DERIVED MESENCHYMAL STEM CELLS IN ROTATOR CUFF 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 this disease. In massive rotator cuff tear than 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 were harvested from the iliac crests of male New Zealand White rabbits weighing 2.0 to 2.5 kg each. A 18-gauge needle were used to penetrate the cortex of the bone and 7~10 ml of marrow were aspirated into a syringe containing 3000 unit of heparin. The bone marrow samples were layered on to Percoll (density 1.073 g/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 1×10^6 per 75 T-flask, and 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. The medium in each plate were replaced with 35 ml of fresh medium on Day 3.

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 2×10^6 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.

Surgical procedure

The rabbit were anesthetized and the shoulder joints were asceptically prepped and both rotator cuff tendons were exposed through 3cm longitudinal skin incisions. Identical, full-thickness, window defects (sized, 5 mm×5 mm) were cut in the central part of each rotator cuff tendon (Fig 1). The right side rotator cuff tendon defect were then grafted with the autologous MSC seeded OPLA scaffold (Fig 2). The left side rotator cuff tendon defect was implanted with the cell-free OPLA scaffold implant and a biodegradable suture, similar to the treated side. Another three rabbits were used for control of tendon defect without treating..

Fluorescent anaysis

At the end of the secondary passage, digital images of the MSCs were taken using fluoresent microscope just before seeding into the scaffold. The rabbits were sacrified and the samples were harvested 2, 4 and 6 weeks after implantion, then fresh frozen for later dissection and evaluation. Digital images were acquired using both fluorescent microscope and Bio-Rad confocal microscope. We stained the DNA in cell nucleus with DAPI (4',6-Diamidino-2-Phenylindole) for confirming the cell labeled with PKH-26.

Histological and Immunohistochemical analysis

The samples were harvested 2, 4 and 6 weeks after implantion and fixed in 10% buffered formalin overnight, washed, and dehydrated through a graded series of alcohol and were embedded in paraffin. Sections 4 mm thick, were obtained from the tendon specimens, and then stained with hematoxylin and eosin, and immunohistochemical stains for collagen type I, II. Slides were counterstained with hematoxylin, and examined by light microscopy to determine type-I and II collagen distribution. For a negative control, the primary antibody was omitted.

Fig. 1. Full-thickness, window defects (sized, 5 mm×5 mm) were cut in the central part of rotator cuff tendon.

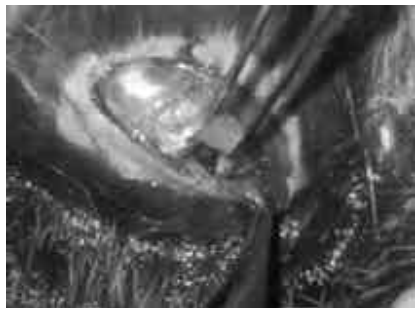

Fig. 2. The defect was grafted with the autologous MSCs seeded OPLA scaffold.

Results

At the end of the second passage, the fluorescently labeled MSCs were identified in the culture media. Numerous cells labeled with PKH-26 were integrated well into the OPLA scaffold following 2 weeks after implanted in the defect of rotator cuff tendon of the rabbit. We confirmed the existence of the living cells labeled with PKH-26 in the OPLA scaffold by staining with fluorescent staining (Fig 4) compared with no cell staining in scaffold (Fig 3). The same characteristics were confirmed at 4 and 6 weeks implantation with the results at 2 weeks implantation (Fig 5, 6)

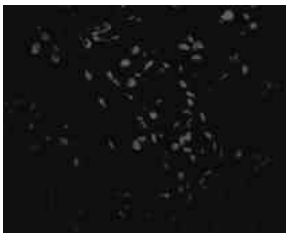

Fig. 3. Scaffold

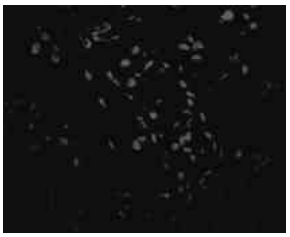

Fig. 4. Scaffold + Stem cell

Fig. 3. No Cell was labeled in red with PKH-26 in OPLA scaffold in vivo at 2 weeks implantation (×100).
Fig. 4. MSCs were labeled in red with PKH-26 in OPLA scaffold in vivo at 2 weeks implantation (×100).

Fig. 5. Scaffold

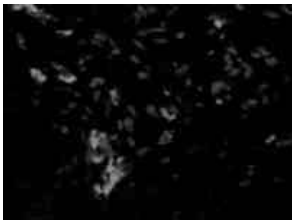

Fig. 6. Scaffold + Stem cell

Fig. 5. No Cell was labeled in red with PKH-26 in OPLA scaffold in vivo at 6 weeks implantation (×100).
Fig. 6. MSCs were labeled in red with PKH-26 in OPLA scaffold in vivo at 6 weeks implantation (×100).

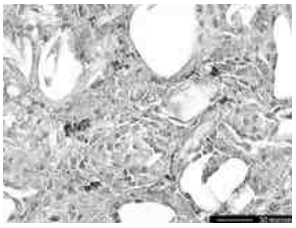

Fig. 7. Scaffold (×100)

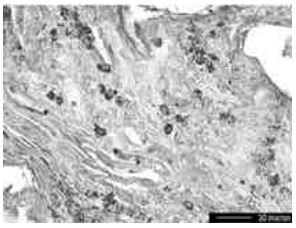

Fig. 8 . Scaffold + Stem cell (×100)

Immunohistochemical staining for collagen I in the scaffold (**Fig. 7**) and scaffold with MSCs (**Fig. 8**) in vivo at 6 weeks implantation. The expression of collagen I was higher in the scaffold with MSCs than in the scaffold without MSCs.

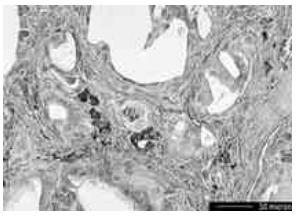

Fig. 9. Scaffold (×100)

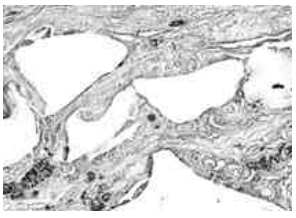

Fig. 10. Scaffold + Stem cell (×100)

Immunohistochemical staining for collagen II in the scaffold (**Fig. 9**) and scaffold with MSCs (**Fig. 10**) in vivo at 6 weeks implantation. The expression of collagen II was not different between the scaffold with MSCs and the scaffold without MSCs.

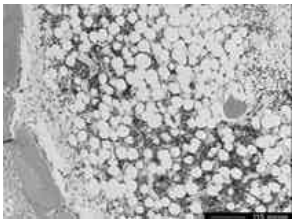

Fig. 11. Ectopic bone (×10)

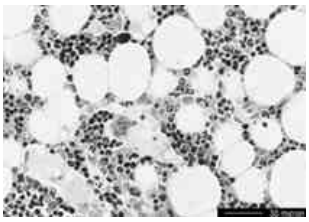

Fig. 12. Ectopic bone (×100)

Hematoxylin-Eosin(HE) staining of ectopic bone formation within the tendon. The cortical bone and fat marrow was developed in the scaffold with MSCs at 6 weeks implantation (**Fig. 11** & **Fig. 12**)

The expression of Immunohistochemical stainings for collagen I was higher in the scaffold with MSCs than in the scaffold without MSCs. The expression of Immunohistochemical stainings for collagen II, however, was not different between the scaffold with MSCs and the scaffold without MSCs (Fig 9, 10). The ectopic bone was incidentally developed in the scaffold with MSCs at 6 weeks implantation.(Fig 11, 12)

Discussion & Conclusion

We 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 promote the tendon healing by producing collagen type I when they were applied at the tendon defect. However, further investigation should be performed to solve the unexpected problems like ectopic bone formation in the tendon. We believe this study would serve as the important step toward the cell therapy of the rotator cuff tendon tear in the shoulder joint.