**Synovial Mesenchymal Stem Cells Enhance Healing of Meniscal Repair In The Avascular Zone of Longitudinal Tear Using A Pig.**

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**Introduction:** Synovium plays a pivotal role in meniscal healing [1], and we previously reported that intra-articular injection of synovial mesenchymal stem cells (MSCs) promoted meniscus regeneration in animal studies [2-4]. The purpose of this study was to examine the effect of transplantation of allogeneic synovial MSCs on healing of the meniscus in a sutured longitudinal tear model of pigs.

**Methods:**

1. **Animals**
   Skeletally mature microminipigs were used. Only one pig was used as a donor for transplantation of allogeneic synovial MSCs, and 13 pigs were used as recipients.

2. **Surgical procedures**
   Full-thickness longitudinal tear was created in the avascular zone from the anterior to the posterior part with use of a 1.0 mm diameter biopsy punch (Fig. 1Aa). The tear was repaired by mattress sutured using nylon treads (Fig. 1Ab). For the MSC group, with the tibial joint surface facing upward, 2×10⁷ synovial MSCs in 100μl of Veen-3G were placed directly into the meniscal lesion (Fig. 1Ac). Knees were then held stationary for ten minutes (Fig. 1Ad). For the control group, the same volume without synovial MSCs was implanted. Medial menisci from both knees were harvested at 2 (n=4), 4 (n=4), and 12 weeks (n=5).

3. **Macroscopic examination**
   To quantify the extent of healing meniscal lesion, the macro score was used as follows; 0: nothing, 1: partial, 2: complete. The lesion was divided into anterior, middle, and posterior part. Each part was scored respectively.

4. **Histological examination**
   The sections at 5μm were stained with toluidine blue, and H&E. Immunohistochemistry for Ki67 was examined. To quantify the meniscal lesion, histological score was used. This score consists of six categories scored on 0 (worst)-18 (best) points. To quantify the extent of synovial tissue induced into lesion, a synovial coverage score was used as follows; 0: nothing, 1: minimal, 2: moderate, 3: marked.

5. **MRI T1rho mapping**
   Sagittal T1rho mapping was taken by 3.0T MRI. The Ziostation II (Ziosoft) was used for the analysis.

6. **Biomechanical analysis**
   After removal of suture threads, meniscus was set on mechanical testing frame (Autograph). The specimens were then split along the lesion with a defined velocity of 1 mm/min. The force needed to separate the meniscus in the repair tissue was recorded. After the test, the sectional area of lesion was measured using stereoscopic microscope (Olympus MVX10).
7. Cell tracking for GFP+ MSCs
Synovial MSCs derived from GFP transgenic microminipig were transplanted. After skin closure, the knee was flexed and extended 100 times, and then it was re-opened to observe.

8. Cell tracking for MSCs labelled with ferucarbotran by MRI
The MSCs were cultured for 24 hours in medium with ferucarbotran (Resovist) and transplanted. The knee joints were examined by MRI at 2 weeks.

9. Statistical analyses
Comparisons between two groups were analyzed using the paired t-test. P-values less than 0.05 were considered to be statistically significant.

**Results:** We demonstrated that cells derived from synovium of a microminipig have properties of MSCs (Fig. 1B, 1C). Macroscopically, in the MSC group at 4 weeks, a part of lesion healed. At 12 weeks, whole lesions healed partially or completely. Contrarily, in the control group, many lesions did not heal even at 12 weeks (Fig. 2A). The macro score in the MSC group was significantly better than that in the control group at 4 and 12 weeks (Fig. 2B). Histologically, at 2 weeks in the MSC group, the lesion healed partially in all cases. In the control group, the lesion healed partially in only one case. At 4 weeks, in the MSCs group, the lesion healed completely in 3 cases and partially in one case. In the control group, the lesion healed partially in two cases. At 12 weeks, in the MSC group, the lesion healed completely in all cases. In the control group, the lesion healed partially in all cases. The lesions still showed less metachromasia compared with the MSC group. (Fig. 3A). The histological score in the MSC group was also significantly better that in the control group at 2, 4 and 12 weeks (Fig. 3B). Transmission electron microscopic (TEM) analysis demonstrated that the lesion in the MSC group at 12 weeks was occupied by dense collagen fibrils with smaller diameter than those observed in an intact meniscus (Fig. 3C). Contrarily, in the control group, organized collagen fibrils were not observed. We also evaluated the effect of transplantation of synovial MSCs on synovium induction into lesion. In the MSC group at 2, 4 and 12 weeks, a large number of synoviocytes along superficial layer were observed from outer zone into lesion in inner zone sequentially. Contrarily, in the control group, the synoviocytes didn’t reach the lesion at 2 and 4 weeks (Fig. 3D). The synovial coverage score in the MSC group was significantly better than that in the control group at 2 and 4 weeks (Fig. 3E). Many synoviocytes induced synovial MSCs were proliferating as shown by Ki67 positive (Fig. 3F; white arrows). TEM of superficial layer for lesion demonstrated that in the MSC group at 4 weeks, spindle-shaped or polygonal cells were observed between loose collagen fibrils. In the control group, a few cells were observed in the superficial layer (Fig. 3G). These findings indicated that synovial MSCs induced synovial tissue into lesion. MRI T1rho mapping was able to evaluate extracellular matrix in cartilaginous tissue [5]. T1rho values of mean anterior and posterior part of menisci in the MSC group was significantly better than those in the control group (Fig. 4A, 4B). In the biomechanical analysis, the tensile strength at failure in the MSC group was higher than that in the control group in all 4 pigs (Fig. 5B). To examine where transplanted cells were adhered just after transplantation, early adhesion assays were performed using GFP+ cells. GFP signals were observed in the lesion and synovium (Fig. 6A; white arrows). To track the cells by MRI, synovial MSCs labelled with ferucarbotran were used. Synovial MSCs included particles of ferucarbotran 24 hours after incubation (Fig. 6B; black arrowheads). At 2 weeks, ferucarbotran in the MSCs were observed around lesions and anterior synovium (Fig. 6C; white arrow).
**Discussion:** The induction of synovial tissue to the meniscal lesion might be an important initiation for meniscal healing. The induction of synovial tissue to the meniscal lesion might be an important initiation for meniscal healing, therefore, several methods were attempted to promote this process [6, 7]. We demonstrated exogenous synovial MSCs enhanced induction of synovium into lesion. This results provide more convincing evidence that synovial MSCs can stimulate healing of meniscal repair. In conclusion, transplantation of allogenic synovial MSCs enhanced healing in sutured torn menisci through promoting induction of synovium into lesion.

**Significance:** Transplantation of synovial MSCs can enlarge indication for sutured repair to meniscus injury. This contributes to increase chance of saving meniscus and prevent from exacerbating osteoarthritis in meniscus injury patients.