Newly-developed Implantation Procedure with Synovium-derived Mesenchymal Stem Cells for Cartilage Regeneration

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Introduction: Mesenchymal stem cells (MSCs) are an attractive cell source for cartilage regenerative medicine because they can be harvested in a minimally invasive manner, are easily isolated and expanded, and have multipotentiality that includes chondrogenesis (1). In addition, synovial MSCs are especially promising due to their high proliferative capacity and chondrogenic potential (2-6). Current cell therapy for cartilage regeneration requires invasive procedures, periosteal coverage and scaffold use. We have developed a novel implantation procedure with synovial MSCs to solve these problems.

Materials and Methods: Full thickness cartilage defects were created in a rabbit’s knee, filled with a cell suspension of ten million Dil-labeled autologous synovial MSCs in 100 μl PBS and left stationary for 10 minutes with the defect upward (local adherent group). As control, either ten million synovial MSCs in 100 μl PBS were injected intra-articularly (intra-articular group), or the defects were left empty (control group). These three groups were compared macroscopically and histologically. The minimum duration for an adequate number of synovial MSCs to attach to the defect was determined through initial ex vivo sequential analysis.

Results: In vivo, in the local adherent group, a large number of implanted synovial MSCs attached to the defect at 1 day, formed a layer 20 cells deep (Fig. 1), and the cartilage defect improved in 24 weeks (Fig. 2A). The histological score of the local adherent group was consistently better than the scores of the other two groups (Fig. 2B). In the sequential ex vivo analysis, the attached cell number increased in a time-dependent manner and reached a plateau in ten minutes (Fig. 3).

Discussion: Based on these results, we summarize the local adhesion technique for clinical application as follows: (A) when the operation for the cartilage injury is performed, (B) the knee is positioned so that the cartilage defect is up. Then, the synovial MSC suspension is slowly dripped onto the cartilage defect and the knee is held stationary for 10 minutes. (C) The knee position is then permitted to be changed and the synovial MSCs are adhered to the cartilage defect. (D) The implanted synovial MSCs differentiate appropriately for the local microenvironment, and the cartilage regenerates. Additionally, this procedure can be performed, without the need for additional scaffold, arthroscopically from the cell harvest to the implantation. This protocol will advance and extend the clinical application of MSC-based cell therapy for cartilage injury.


Fig. 1 Histological observation at 1 day after cell transplantation. Sagittal sections stained with Toluidine Blue and the serial sections under fluorescence for the Dil-label are shown. Higher magnifications of the framed areas were shown in panel E, F, I and J. The nuclei were counterstained by DAPI in panel F and J.

Fig. 2 Histological observation. A, sagittal sections stained with Toluidine Blue. Distal side is shown on right side of the image. B, histological score for the cartilage defect after cell transplantation. Histological findings were quantitated using the scoring system, in which a full score was 14 and a higher score indicates cartilage repair. The scores of the local adherent group were better than other groups at each point. The data are expressed as mean ± standard deviation (n=3; *p<0.05).

Fig. 3 Ex vivo sequential analysis of the cell number attached to the cartilage defects by the local adherent technique. A, scheme for the method. a, the cartilage defect of the femoral condyle was faced upward and the defect was filled with Dil-labeled rabbit synovial MSC suspension. b, the defect was held stationary for 2.5, 5, 7.5, 10, and 15 minutes. c, the femur was turned with the defect side down for 10 minutes so that “non-adhered cells” in the defect fell in the culture medium. Then, non-attached cell number was determined, and the attached cell number extrapolated. B, the cell number attached to the cartilage defects by the local adherent technique. The data are expressed as mean ± standard deviation (n=3). *Significantly different from 2.5 min, # significantly different from 5 min (Mann-Whitney U test; p<0.05).