

M2 macrophages in adipose-derived stromal vascular fraction cells contribute to anabolic-promoting and catabolic-inhibitory effects in osteoarthritis

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INTRODUCTION: Articular cartilage has limited potential for self-regeneration, and damage to articular cartilage ultimately leads to the development and progression of osteoarthritis (OA). Cell transplantation strategies such as mesenchymal stromal cells (MSCs) have emerged as a novel therapeutic approach to regenerate articular cartilage¹. Adipose-derived stromal vascular fraction (SVF) cells have attracted attention because they have the comparable multilineage potential as bone marrow MSCs and can be easily obtained in large numbers from subcutaneous adipose tissue without culturing, making them easy to apply clinically². Previous studies have reported that the paracrine effect of SVF cells may be effective in regenerative therapies such as nerve regeneration and fracture healing^{3,4}. Several reports have been published on the clinical application of SVF cells⁵, however, their role in OA therapeutic strategies is still unclear. The purpose of this study is to evaluate the therapeutic efficacy of intra-articular injection of human adipose-derived SVF cells in immunodeficient rat OA models.

METHODS: SVF was isolated from human abdominal subcutaneous fat. OA in rats was induced surgically by destabilization of the medial meniscus (DMM), and 1×10^5 SVF cells, 1×10^4 adipose-derived stem cells (ADSCs), or phosphate-buffered saline (PBS) were injected into the knee joint after the DMM procedure. Rats were sacrificed after 4 and 8 weeks of DMM and compared histologically and immunohistochemically between the groups. In the *in vitro* study, CD163-positive cells in human SVF were analyzed by flow cytometry and sorted as M2 macrophages (M2Φ). In tubes, 1×10^5 human chondrocytes were co-cultured with 1×10^5 SVF cells, 1×10^4 M2Φ, 1×10^4 ADSCs or PBS. After 48 hours, supernatants were analyzed for TGF-β and IL-10 levels using ELISA. After 3 weeks, pellet sizes were evaluated and stained by safranin O-fast green.

RESULTS: Histological assessment using safranin O-fast green staining showed obvious loss of staining, erosions, and decrease of chondrocyte density in the control group, however, cartilage was best retained in the SVF group, and some cartilage remained in the ADSC group (Fig.1a). Hematoxylin and eosin staining showed an increased number of lining cell layers, proliferation of subsynovial tissue, and infiltration of inflammatory cells in the synovium of the control group, while the SVF group had a thinner lining cell layer and less subsynovial tissue proliferation (Fig.1b). Fluorescent immunohistochemical staining of rat synovial tissue showed that the ratio of CD163 expression in the F4/80 positive area was higher in the SVF group than in the control or ADSC groups. In addition, the SVF group showed expression of hNA in CD163-positive area (Fig.2). In the *in vitro* analysis, flow cytometry determined 15.8% of SVF cells were CD163-positive (Fig.3a). In ELISA, both TGF-β and IL-10 levels were highest in the SVF group, followed by the M2Φ group, which were significantly higher than ADSC and control groups, respectively (Fig.3b). Pellet size was also largest in the SVF group, followed by the M2Φ group (Fig.3c).

DISCUSSION: In our study, OA progression and synovial inflammation were clearly milder in the SVF group than in the control group in histological and immunohistochemical evaluation. The results indicate that intra-articularly injected SVF cells regulated the progression of cartilage degeneration and inhibited inflammation of the synovium in OA. This may be caused by homing to the synovium and secretion of fluid factors with chondroprotective effects, including modulation of chondrocyte viability and cartilage matrix protection. Additionally, the SVF group was more effective in treating OA than the ADSC group, potentially due to the prominent M2Φ presence. Fluorescent immunostaining showed the highest expression of M2Φ on synovial tissue in the SVF group, and only SVF group exhibited hNA-positive CD163-positive cells, suggesting that human-derived M2Φ in SVF cells contributed to the suppression of rat synovitis. Furthermore, *in vitro* experiments indicated that M2Φ may increase the expression of TGF-β and IL-10 and promote chondrogenesis. The paracrine effects of SVF cells, possibly from anti-inflammatory macrophages, could enhance its efficacy in promoting chondrogenesis and inhibiting synovitis.

SIGNIFICANCE: Considering our findings, intra-articular injections of SVF cells could be effective in the treatment of OA. SVF cells are less invasive and quicker to isolate than other MSCs, making them easier to use clinically, so we recommend their use as an alternative to other MSCs for OA treatment.

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Figures:

