# Mesenchymal stem cells in synovial fluid increase in number in response to synovitis and display more tissue-reparative phenotypes in osteoarthritis

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**Disclosures**: All authors (N)

**INTRODUCTION**: Synovial fluid mesenchymal stem cells (SF-MSCs) originate in the synovium and contribute to the endogenous repair of damaged intra-articular tissues. Here, we clarified the relationship between their numbers and joint structural changes during osteoarthritis (OA) progression and investigated whether SF-MSCs had phenotypes favorable for tissue repair, even in an OA environment.
**METHODS**: Partial medial meniscectomy (pMx) and sham surgery were performed on both knees of rats. SF and knee joints were collected from intact rats and from rats at 2, 4, and 6 weeks after surgery. SF was cultured for 1 week to calculate the numbers of colony-forming cells and colony areas. Joint structural changes were evaluated histologically to investigate their correlation with the numbers and areas of colonies (Fig.1 A). RNA sequencing was performed for SF-MSCs from intact knees and knees 4 weeks after the pMx and sham surgery. All animal care and experiments were conducted in accordance with the institutional guidelines of the Animal Committee of Tokyo Medical and Dental University (permission number: A2021-267A) and the ARRIVE guidelines.
**RESULTS**: Colony-forming cell numbers and colony areas were greater in the pMx group than in the intact and sham groups and peaked at 2 and 4 weeks, respectively (Fig.1 B and C). Synovitis scores showed the strongest correlation with colony numbers (R = 0.583) and areas (R = 0.456) (Fig.2 A). The finding that synovitis was most closely associated with colony number and area prompted us to perform immunostaining for synovitis-related and MSC mobilization–related molecules (CD68, CD73, and ZO-1). CD68-positive area of synovium correlated with colony number and area (Fig.2 B). RNA sequencing revealed higher expression of genes related to cell proliferation in SF-MSCs in the pMx group than in the intact group and higher expression of genes related to extracellular matrix binding, TGF-β signaling, and superoxide dismutase activity in SF-MSCs in the pMx group than in the sham group (Fig.3 A and B).
**DISCUSSION**: This study revealed that the number of SF-MSCs was most closely correlated with the severity of synovitis in a rat OA model. Immunostaining of the synovium revealed a correlation between the areas positive for CD68 (a macrophage marker) and the changes in the number and colony area of SF-MSCs. Our findings suggest that synovial macrophages regulate the mobilization of synovial MSCs into the SF in OA. The expression of tissue-reparative genes was greater in SF-MSCs from OA knees than in SF-MSCs from knees with no structural joint damage. The present finding that SF-MSCs showed tissue reparative phenotypes even in an OA environment supports the investigation of therapeutic strategies that could recruit endogenous synovial MSCs into the SF to promote joint regeneration. We used culture-expanded MSCs for RNA sequencing because uncultured SF-MSCs are rare in intact knees. However, in vitro culture is known to affect gene expression profiles. Single-cell RNA sequencing will be a promising solution.
**SIGNIFICANCE/CLINICAL RELEVANCE**: SF-MSCs are considered to play a significant role in the endogenous repair of intra-articular tissues. An understanding of how SF-MSCs in OA knee joints change their numbers and phenotypes is critical for developing OA treatments that do not require the application of exogenous MSCs.

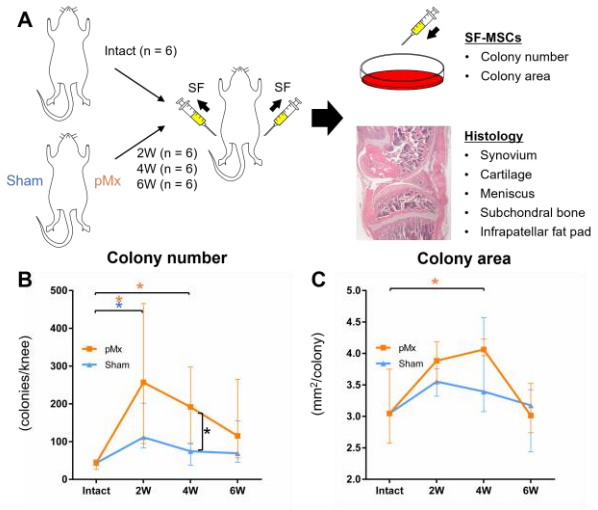
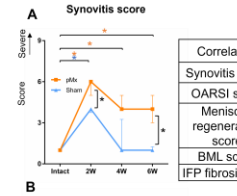
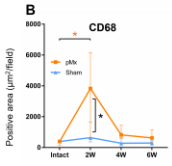



**Fig.1** Changes in the numbers of colony-forming cells in synovial fluid (SF) and their colony areas in rat OA model




| | Correlation with colony number | | Correlation with colony area | |
|---|---|---|---|---|
| Correlation | R | P value | R | P value |
| Synovitis score | 0.583 | <0.001 | 0.456 | 0.02 |
| OARSI score | 0.477 | 0.01 | 0.289 | 0.44 |
| Meniscus regeneration score | -0.475 | 0.01 | -0.434 | 0.03 |
| BML score | 0.375 | 0.07 | 0.18 | 1 |
| IFP fibrosis area | 0.375 | 0.10 | 0.201 | 1 |




| | Correlation with colony number | |
|---|---|---|
| Correlation | R | P value |
| CD68-positive area | 0.476 | 0.001 |
| CD73-positive area | 0.110 | 0.489 |
| ZO-1-positive area | -0.253 | 0.106 |

**Fig.2** Histological evaluations and their association with the numbers and areas of colonies

**A Upregulated genes (pMx vs. intact)**

| GO process | Q value |
|---|---|
| Immune system process | <0.0001 |
| Chemotaxis | <0.0001 |
| Neutrophil chemotaxis | <0.0001 |
| **Positive regulation of ERK1 and ERK2 cascade** | <0.0001 |
| Macrophage chemotaxis | 0.0004 |
| Positive regulation of neutrophil chemotaxis | 0.0004 |
| Integrin-mediated signaling pathway | 0.0004 |
| Inflammatory response | 0.0006 |
| Immune response | 0.0006 |
| Response to lipopolysaccharide | 0.0008 |
| Positive regulation of natural killer cell chemotaxis | 0.0009 |
| **Positive regulation of cell proliferation** | 0.0013 |

**Upregulated genes (pMx vs. sham)**

| GO function | Q value |
|---|---|
| **Extracellular matrix binding** | 0.0005 |
| **Heparin binding** | 0.0008 |
| **Type III transforming growth factor beta receptor binding** | 0.0018 |
| **Superoxide dismutase activity** | 0.0039 |
| **Laminin binding** | 0.0039 |
| **Type II transforming growth factor beta receptor binding** | 0.0044 |
| **IgG binding** | 0.0136 |
| **Integrin binding** | 0.0138 |
| Scavenger receptor activity | 0.0154 |
| Extracellular matrix structural constituent | 0.0178 |

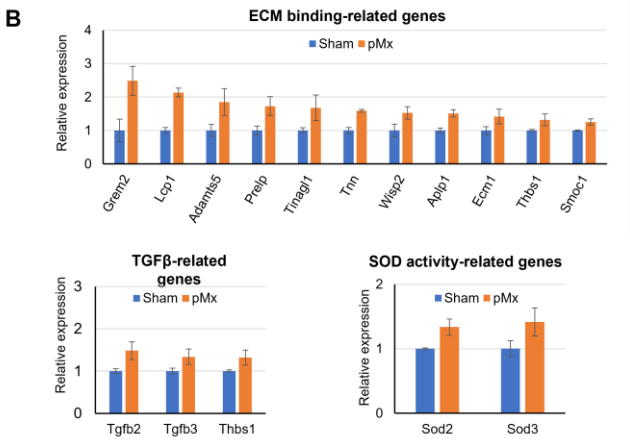



**Fig.3** RNA sequencing of colony-forming cells in synovial fluid.