

RESEARCH ARTICLE

Human adipose and synovial mesenchymal stem cells improve osteoarthritis in rats by reducing chondrocyte reactive oxygen species and inhibiting inflammatory response

Xunzhi Liu¹  | Yaqing Liu² | Huabin He¹ | Weiwei Xiang¹ | Cheng He¹

¹Orthopedics Department, First Affiliated Hospital of Gannan Medical University, Ganzhou City, China

²Pediatric Department, First Affiliated Hospital of Gannan Medical University, Ganzhou City, China

Correspondence

Xunzhi Liu, Orthopedics Department, First Affiliated Hospital of Gannan Medical University, No. 23 Qingnian Road, Zhanggong District, Ganzhou City, Jiangxi Province 341000, China.
Email: liuxunzhi_xzhl@163.com

Funding information

This work was supported by the Science and Technology Research Project of Education Department of Jiangxi Province - Youth Project [GJJ180821].

Abstract

Background: We explored the therapeutic effects of Adipose-derived mesenchymal stem cells (ADMSCs) and Synovial-derived mesenchymal stem cells (SDMSCs) on osteoarthritis (OA).

Methods: SDMSCs and ADMSCs were co-cultured with chondrocytes and stimulated with interleukin (IL)-1 β . An OA model was established on rats by intra-articular injection with ADMSCs and SDMSCs. After 8 weeks, the joint diameter difference was detected, and histological staining was used to observe the pathological changes in cartilage tissue. Enzyme-linked immunosorbent assay (ELISA) was used to detect the expressions of IL-6, tumor necrosis factor (TNF)- α and IL-1 β in joint fluid. The expressions of COL2A1, Aggrecan, Matrix metalloproteinase (MMP)-13, SOX9, IL-6, TNF- α and IL-1 β were detected by qRT-PCR and Western blotting in cartilage tissue. Reactive oxygen species (ROS) content in cells and cartilage tissues was detected by ROS kit.

Results: SDMSCs and ADMSCs co-cultured with chondrocytes could reduce MMP-13 expression, increase the expressions of COL2A1, Aggrecan and SOX9, as well as reverse the effects of IL-1 β on promoting ROS content and inflammatory factors levels. After the OA model was established, the injection of ADMSCs and SDMSCs reduced the differences in joint diameter and tissue lesions in OA rats. The OA model led to increased levels of IL-6, TNF- α and IL-1 β in joint fluid and cartilage tissue, while the injection of ADMSCs and SDMSCs inhibited the inflammatory factor levels in OA rats, and increased the expressions of COL2A1, Aggrecan and SOX9 in OA rats.

Conclusion: ADMSCs and SDMSCs improve osteoarthritis in rats by reducing chondrocyte ROS and inhibiting inflammatory response.

KEYWORDS

adipose-derived mesenchymal stem cells, chondrogenic factors, inflammatory factors, osteoarthritis, synovial-derived mesenchymal stem cells

Xunzhi Liu and Yaqing Liu contributed equally to this work.

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial-NoDerivs](https://creativecommons.org/licenses/by-nc-nd/4.0/) License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2022 The Authors. *Journal of Clinical Laboratory Analysis* published by Wiley Periodicals LLC.

1 | INTRODUCTION

Osteoarthritis (OA) is a chronic joint disease characterized by the destruction of articular cartilage integrity, causing pain and degeneration of the articular cartilage layer, and may be accompanied by changes in bone and cartilage margins.¹ With the intensification of the aging population, the incidence of OA has increased year by year, and OA has developed into a prominent social problem.² At present, the etiology and pathogenesis of OA have yet to be clarified. The clinical treatment of OA mainly includes the use of painkillers, non-steroidal anti-inflammatory drugs, and the addition of lubricating supplements to joint injuries, as well as joint replacement surgery in the late stage of OA.³⁻⁵ However, these methods can only reduce the pain of OA patients and delay the progress of OA, but cannot treat OA fundamentally and control its incidence effectively. Mesenchymal stem cells (MSCs) are resident pluripotent cells existing in adult tissues such as bone marrow, adipose tissue and umbilical cord. By dint of the multi-lineage differentiation ability to differentiate into a variety of adult cells such as fat, bone, cartilage cells, etc., and the capacity of secreting the trophic factors with regenerative function, MSCs are considered to be the ideal candidate cells for cartilage regeneration, and now have been applied to inflammatory diseases.^{6,7}

Mesenchymal stem cells derived from various sources have been reported to be used as seed cells for cartilage defects of early OA in the knee joint.^{8,9} Besides, different sources of MSCs have their own different advantages and disadvantages. Bone marrow (BM) is the most common source of MSCs, but BMMSCs are difficult to obtain and are prone to cause donor site-induced diseases; moreover, they also face the problems of aging and decreased proliferation and differentiation abilities with the increase in the number of passages.¹⁰ Compared with BMMSCs, adipose-derived MSCs (ADMSCs) are much easier to obtain and have strong proliferation and differentiation potential, good anti-inflammatory effect, but poor osteogenic and chondrogenic potential.¹¹ Compared with MSCs from other sources, synovial-derived MSCs (SDMSCs) have stronger chondrogenic differentiation abilities, and their multiple passages *in vitro* do not show signs of cell degeneration.^{12,13} MSCs from other sources, such as umbilical cord MSCs, are difficult to separate, and their potential to treat OA is limited to pre-clinical researches.¹⁴ As the availability of MSCs and the ability of chondrogenic differentiation are mostly considered in the clinical application, the combined application of ADMSCs and SDMSCs may exert their respective advantages to better repair the regenerated cartilage tissue.

However, the mechanism of the combined application of ADMSCs and SDMSCs in the treatment of OA has not yet been expounded. Therefore, this research endeavored to obtain human ADMSCs and SDMSCs, combine application of ADMSCs and SDMSCs to treat OA rats, and observe its clinical treatment effect on OA rats, hoping to provide a new clinical reference for the treatment of OA.

2 | METHODS

2.1 | Ethics statement

The animal experiments in this study were approved by the Animal Ethics Committee of First Affiliated Hospital of Gannan Medical University (FAHGMU20190125) and were conducted in accordance with the guidelines of the National Institutes of Health (USA) for animal experiments.

In this study, the experiments were approved by the Animal Ethics Committee of the First Affiliated Hospital of Gannan Medical University, and strictly abided by the statement of experimental principles concerning the welfare and ethics based on the guidelines of the National Institutes of Health.

2.2 | Isolation of SDMSCs and ADMSCs

Samples of the synovial tissue in joint and infrapatellar fat pad (IPFP) were harvested from OA patients aged 50–60-year-olds in the First Affiliated Hospital of Gannan Medical University, including 10 male patients and 10 female patients. All the cases met the 1986 American College of Rheumatology (ACR) criteria for classification and reporting of knee OA. These donors suffered from rheumatic diseases and infections and had a history of malignancy prior to study were excluded. All patients provided the written informed consents.

The adipose tissues (approximately 6 g) under the IPFP of patients undergoing knee arthroscopy surgery were repeatedly rinsed 10 times with the tissue wash solution containing 1% penicillin, and then cut straight into a paste. Next, the paste-like tissues were transferred to a 15-ml centrifuge tube containing 0.2% collagenase XI (Sigma Shanghai) and incubated on a 37°C shaker for 1 h, followed by a continue incubation with 0.1% trypsin (Sigma Shanghai) for 30 min. Subsequently, the tissues were filtered with a 200-mesh filter. Then, the filtrate was collected, put into a 50-ml centrifuge tube, and centrifuged at 1000 × g for 5 min. After the supernatant was discarded, the remaining samples were resuspended in Phosphate buffered saline (PBS) and centrifuged at 1000 × g for 5 min. Following the removal of the supernatant, Dulbecco's Modified Eagle Medium (DMEM) (Gibco) containing 10% Fetal Bovine Serum (FBS) was added to resuspend the samples, subsequent to which the samples were inoculated into the 6-well plates. Through further cultivation in a cell incubator at 37°C with 5% CO₂, ADMSCs were obtained from the samples. When the cell confluence reached about 70%, the cells were subject to digesting and passaging. After continuous culture, the second-generation cells were singled out for subsequent experiments.

With regard to the isolation and culture of SDMSCs, 5 g of synovial tissues were taken from the supraclavicular capsule of the knee joint by arthroscopic surgery, and then, the collected surgical specimens were quickly immersed in sterile PBS at 4°C. Thereafter, the surgical specimens were washed 10 times with sterile PBS buffer to remove fat and other connective tissue components, and to separate

the bright synovial tissues. SDMSCs were harvested by enzymatic separation using the same method as ADMSCs.

2.3 | Cell grouping

The second-generation articular cartilage cells were divided into seven groups: Control group (the articular cartilage cells were cultured in the normal medium), IL-1 β group (the articular cartilage cells were cultured in the medium and treated with IL-1 β), co-culture + SDMSCs group (the articular cartilage cells and SDMSCs were co-cultured in the medium and treated with IL-1 β), co-culture + ADMSCs group (the articular cartilage cells and ADMSCs were co-cultured in the medium and treated with IL-1 β), co-culture + SDMSCs + ADMSCs group (the articular cartilage cells, SDMSCs and ADMSCs were co-cultured in the medium and treated with IL-1 β), SDMSCs group (SDMSCs were cultured in the normal medium) and ADMSCs group (ADMSCs were cultured in the normal medium). For IL-1 β treatment, cells were treated with 25 ng/ml IL-1 β (Thermo Fisher Scientific, Waltham, MA, USA) for 48 h.

2.4 | Isolation of chondrocytes

Sprague–Dawley (SD) rats (6-week-old, 180 g–200 g) were provided by Hunan Slake Jingda Laboratory Animal Co., Ltd. (<http://www.hnsja.com/>). Chondrocytes were isolated from the articular cartilage tissues of rats for primary culture. Briefly, after the rats were anesthetized with 10% sodium pentobarbital, the rat articular cartilage was taken under aseptic conditions and washed twice with PBS (100 U/ml) containing penicillin and streptomycin to remove the periosteum and fibrous tissue on the cartilage surface. Next, the cartilage was cut into 1 mm \times 1 mm tissue fragments, added with 3 times the volume of 0.25% pancreatin and then digested at 37°C for 0.5 h. After the trypsin was discarded, DMEM containing 15% FBS was added to terminate the digestion, followed by the centrifugation at 1000 \times g for 5 min. When the cell supernatant was discarded, 2 times the volume of 0.1% type II collagenase was added in the cell suspension for 3.5-h digestion. Later, the cell suspension was extracted and underwent the observation with the help of a microscope. After the cell volume became sufficient, the cells were filtered through a 200-mesh metal screen. After centrifugation for 10 min, the cell supernatant was discarded, and the remaining was washed twice with medium. Then, the cells were resuspended by adding 5 ml of complete medium (containing 15% FBS, penicillin 100 U/ml, streptomycin 100 g/ml). Afterward, the cells were seeded in a 25-ml culture flask and cultured, and labeled as primary cells. After the articular cartilage cells were cultivated with the second generation of chondrocytes, the cells were taken for co-culture with MSCs.

2.5 | Animal grouping

A total of 40 male SD rats (6-week-old, 180 g–200 g) were provided by Hunan Slake Jingda Laboratory Animal Co., Ltd. (<http://www.hnsja.com/>).

The experimental rats were randomly assigned into 5 groups, namely Sham group ($n = 8$), OA group ($n = 8$), OA + ADMSCs group ($n = 8$), OA + SDMSCs group ($n = 8$) and OA + SDMSCs + ADMSCs group ($n = 8$). The experimental rats were placed in cages with standard temperature, given 12:12 light/dark cycle conditions, and given free movement, standard diet and water.

2.6 | Establishment of a rat OA model

For OA group, the OA model of rats was established by Hulth method, and rats were fasted for 12 h before modeling. After anesthetization with 3% pentobarbital sodium intraperitoneally, the rats were placed on the operating table in a supine position, the mouse hair around the right hind limb knee joint was removed, and the skin of surgical site was routinely sterilized with an alcohol cotton ball. Next, a sterile surgical blade was used to cut the knee joint from the medial side of the patella to expose the knee joint, and then the medial collateral ligament was cut and the joint cavity was opened. Afterward, the patellar ligament was opened and the anterior and posterior cruciate ligaments under surgical vision were removed, to prevent late healing. Subsequently, the parallel drawer experiments were conducted to confirm whether the anterior and posterior cruciate ligaments were cut. Later, the medial meniscus was removed to reset the patellar ligament. During the operation, no extra damage was made to the joint surface, which must be ensured. After complete hemostasis, the incision was sutured layer by layer with a disposable absorbable surgical suture. Post operation, the rats were allowed to move free without fixation of the knee joint on the surgical side. At the same time, to prevent infection, all rats received intramuscular injection of 200,000 units of penicillin every day for 3 days. One week after the operation, exercise training was performed on rats in each group, with a small animal rotating rod instrument to increase the weight of the affected limbs of OA rats. For Sham group, the joint cavity of the rats was opened using the same surgical method by intra-articular injection of 50 μ l PBS solution without any resection, and then the wound was sutured. For OA + ADMSCs group, 12 weeks after surgery, each rat was intra-articularly injected with ADMSCs (3.0×10^5 cells) dissolved in 50 μ l PBS solution. The cell dose of 300,000 cells per joint in rats was referred to the previous research.¹⁵ Likewise, 12 weeks after surgery, the rats in OA + SDMSCs group were subjected to intra-articular injection of SDMSCs (3.0×10^5 cells) dissolved in 50 μ l PBS solution. For OA + SDMSCs + ADMSCs group, 12 weeks after surgery, each rat was injected with 100 μ l of ADMSCs (1.5×10^5 cells) and SDMSCs (1.5×10^5 cells) dissolved in 50 μ l PBS solution. The rats were injected once a week for a total of 8 times. The activity, diet and wound healing of rats were recorded every day, and the weight of rats was measured every week. After 8 weeks of administration, the rats were sacrificed, and the knee joint tissue and joint cavity fluid samples were collected and analyzed. The femoral medial condyle articular cartilage tissue was collected for pathological examination and determination on expression of inflammatory cytokine.

2.7 | Joint diameter difference detection

The activities and diet of the rats as well as wound healing were recorded and observed every day after the operation. For the sutures at the incision, the joint cavity of the rats should be disinfected with iodophor and re-stitched to prevent infection. The weight of the rats was measured and recorded every week. After the rats were sacrificed, each rat's knee joint and the contralateral knee joint (non-operative side) were measured with a caliper, and the difference in knee joint diameter was calculated with the following formula: Knee diameter difference (MM) = diameter of the knee joint on the operation side - diameter of the contralateral knee joint.

2.8 | Reactive oxygen species (ROS) content detection

Initially, the articular cartilage cells were treated in DMEM for 24 h. After being washed with phenol-free red PBS twice, the cells were incubated with 10 μ M of 2',7'-Dichlorofluorescein diacetate (DCFDA) (Cat.#D6883, Sigma) for 30 min. Then, the cells were placed in an incubator at 37°C and additionally incubated with PBS for 1 h. Finally, ROS level in the cells was measured using a microplate reader (Meigu Molecular Instruments (Shanghai) Co., Ltd). The rat cartilage tissues were made into the 5 μ m sections and the content of ROS in all treated rat cartilage tissues was detected in the same way.

2.9 | Hematoxylin and eosin (HE) staining

Cartilage tissues of medial femoral condyle of rats were placed in 4% formaldehyde solution for 24 h, and then decalcified with 15% ethylenediamine tetraacetic acid (EDTA) decalcification solution. The decalcification was regularly checked with acupuncture samples, when the result indicated that the decalcification was complete, the decalcification was terminated. The tissues were washed with running water, dehydrated by alcohol, and transparentized using xylene. Subsequently, the tissues were dewaxed and embedded, and then made into 5- μ m-thick tissue sections. After that, the tissue sections were stained with the hematoxylin and eosin staining kit (C0105, Beyotime Biotechnology). In short, the tissue sections were stained with hematoxylin staining for 5 min, underwent the differentiation using hydrochloric acid ethanol for 30 s, and then further received eosin staining for 2 min. Afterward, the tissue sections were dehydrated, transparentized and mounted, subsequent to which the pathological changes of bone tissue were observed under the microscope (CKX31, Olympus, Japan) at a magnification \times 100.

2.10 | Safranin-O-fast green staining

Cartilage tissues of the medial femoral condyle of rats in each group were subjected to Safranin-O-fast green staining. In a nutshell, the

preparation of tissue paraffin sections was carried out, which was the same as that of HE staining. Then, the tissue sections were stained with hematoxylin for 5 min, washed with distilled water for 10 min and further stained with Fast Green FCF (2353-45-9, Sigma-Aldrich, USA) solution for 5 min, followed by being washed with 1% acetic acid solution (A6283, Sigma-Aldrich, USA) for 10 s and distilled water for 10 min. Afterward, the tissue sections were then stained in 0.1% safranin O solution (TMS-009, Sigma-Aldrich, USA) for 30 min. Later, the tissue sections were dehydrated by gradient alcohol, transparentized using xylene, and sealed with neutral gum. Finally, the stained tissue sections were observed under an optical microscope (CKX31, Olympus, Japan) at a magnification \times 100.

2.11 | Enzyme-linked immunosorbent assay (ELISA)

The joint fluid samples of rats were collected to detect the content of IL-6, tumor necrosis factor (TNF)- α and IL-1 β using IL-6, TNF- α and IL-1 β ELISA detection kits (PI328, PT516, PI303, Beyotime Biotechnology, China), respectively. The joint fluid samples were added into ELISA plates and incubated at room temperature for 2 h. After being washed 5 times with washing solution, each well of the plate was supplemented with 100 μ l of diluted detection antibody, followed by the incubation at room temperature for 1 h. Thereafter, the plate was added with washing solution and rinsed 3 times, and 100 μ l of diluted horseradish peroxidase-labeled streptavidin was put into each well to incubate the plate at room temperature for 30 min. Afterward, 100 μ l coloring substrate 3,3',5,5'-Tetramethylbenzidine (TMB) solution (P0209, Beyotime Biotechnology, China) was added to each well and incubated the plate at room temperature for 20 min in the dark, and then 50 μ l stop solution was applied to stop the color reaction. Finally, the microplate reader (SPECTROstar[®] Nano, BMG LABTECH, USA) was used to detect the optical density (OD) value of each well at the wavelength of 450 nm and 570 nm.

2.12 | Western blotting

The cartilage tissues were collected and treated with lysis buffer (R0278, Sigma-Aldrich, USA). The lysates were centrifuged at 12000 r/min for 5 min at 4°C. Next, the supernatant was collected in a new tube and placed on ice, and the precipitate was discarded. QuantiPro[™] bicinchoninic acid (BCA) detection kit (QPBCA, Sigma-Aldrich, USA) was used to measure the protein concentration. When the protein concentration was 50 μ g/ml, the extracted protein was used for subsequent studies. The proteins were separated by using 15% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Thermo Fisher Scientific, MMAS, US), and then transferred onto nitrocellulose membranes (Thermo Fisher Scientific, MMAS, US). After being sealed by 5% milk at 25°C for 1 h, the membranes were incubated with the primary antibodies against COL2A1 (ab34712, 142 kDa, 1:1000, Abcam, USA), Aggrecan (ab36861, 110 kDa, 1:1000, Abcam, USA), Matrix metalloproteinase (MMP)-13 (ab39012, 54 kDa,

1:1000, Abcam, USA), SOX9 (ab185230, 70 kDa, 1:1000, Abcam, USA), IL-6 (ab9324, 21 kDa, 1:1000, Abcam, USA), TNF- α (ab66579, 26 kDa, 1:1000, Abcam, USA), IL-1 β (ab200478, 31 kDa, 1:1000, Abcam, USA) and Glyceraldehyde-3-phosphate dehydrogenase (GAPDH; ab8245, 36 kDa, 1:1000, Abcam, USA) at 4°C overnight, respectively. Thereafter, the membranes were ulteriorly cultivated with the secondary antibody (Protein tech, USA) for 2 h, and then washed by PBS for three times. Ultimately, the protein bands were measured by the Efficient chemiluminescence (ECL) kit (D3308, Beyotime Biotechnology, China) and then scanned by a super sensitive multifunctional imager (Amersham Imager 600, General Electric Company, USA).

2.13 | Quantitative reverse transcription-polymerase chain reaction (qRT-PCR)

The total RNA from cartilage tissues and cells in each treatment group was extracted with Trizol reagent (R0016, Beyotime Biotechnology, USA) at 25°C. Then, RNA precipitation was dissolved in water, which needed to be quickly operated on ice. Agarose gel electrophoresis was applied to test the integrity of the RNA. If there were 3 bands, it indicated that the RNA was of good integrity. Next, the purity of RNA was detected through the NanoDrop™ One/OneC Ultra-micro UV Spectrophotometer (ND-ONEC-W, Thermo Scientific™, USA). If the OD260/OD280 of RNA was close to 2.0, the RNA purity was high. Subsequently, the RNA concentration was determined and calculated according to the following formula: RNA concentration = OD260 \times dilution factor \times 0.04 μ g/ μ l. When the concentration of RNA reached 500 ng/ μ l, the RNA could be used for subsequent experiments. Then, PrimeScript RT kit (RR037A, Takara, China) was used to reverse-transcribe the RNA into

complementary DNA (cDNA). The mRNA expression levels were quantitated by SYBR Green PCR Master Mix (D7268 M, Beyotime Biotechnology, USA). PCR amplification system was prepared as follows: 2 μ l of nuclease-free water, 5 μ l of 2 \times SYBR Green master mix, 1 μ l of cDNA, 0.5 μ l of forward primer and 0.5 μ l Reverse Primer were mixed together. QRT-PCR was conducted in the ABI7500 system (Applied Biosystems), with the PCR cycle system being set as follows: pre-denaturation at 95°C for 10 min; PCR at 95°C for 3 s and 60°C for 30 s, for a total of 40 cycles. $2^{-\Delta\Delta CT}$ method was employed to calculate the relative mRNA expression levels.¹⁶ All primer sequences of all genes undergoing the detection of qRT-PCR were listed in Table 1.

2.14 | Statistical analysis

The data were analyzed by SPSS 18.0 (Chicago, USA). In this study, measurement data were shown as mean \pm standard deviation. Group differences were measured by one-way analysis of variance, and *t* test was used to compare the difference of the data between two groups. Each treatment was carried out in triplicate. *p* < 0.05 was regarded as statistical significance.

3 | RESULTS

3.1 | Effects of cell co-culture on chondrogenesis, oxidative stress and inflammatory factors

MMP-13 may be a particularly critical enzyme for degrading type II collagen in articular cartilage.¹⁷ COL2A1, Aggrecan and SOX9 are the major markers of cartilage differentiation in BMMSCs.¹⁸ Thus,

TABLE 1 Primers used in real-time PCR analysis

Gene	Primer sequence	Species
COL2A1	Forward: 5'- CCCCAGGTTCTAATGGCAA-3' Reverse: 5'- GGTTGCGCTTTCTCTCCAGG-3'	Rat
Aggrecan	Forward: 5'- CCCTACCCTTGCTTCTCCA-3' Reverse: 5'- CTTGAGAGGCACTCATCAATGT-3'	Rat
MMP-13	Forward: 5'- CTGTGGCTCCTCTAAACCTC-3' reverse: 5'- GAGCACATCCGGCACCTGTA-3'	Rat
SOX9	Forward: 5'- GCCACCGAACAGACTCACAT-3' Reverse: 5'- CCTGAGATTGCCCGGAGTG-3'	Rat
IL-6	Forward: 5'- CCAGTTGCCTTCTTGGGACT -3' Reverse: 5'- TGCCATTGCACAACCTTTTC -3'	Rat
TNF- α	Forward: 5'- GATCGGTCCCAACAAGGAGG -3' Reverse: 5'-CTTGGTGGTTTGCTACGACG-3'	Rat
IL-1 β	Forward: 5'- TCGGAATGTGTCTTGGGCA -3' Reverse: 5'- AGTGATGTTAACCTCCTCCAGC -3'	Rat
GAPDH	Forward:5'- AGGTCGGTGTGAACGGATTTG-3' Reverse:5'- GGGGTCGTTGATGGCAACA-3'	Rat

we detected the expressions of COL2A1, Aggrecan, SOX9 and MMP-13 in this study, and found that SDMSCs and ADMSCs co-cultured with chondrocytes could prominently diminish the expression of MMP-13 and elevate the expressions of COL2A1, Aggrecan and SOX9. When SDMSCs and ADMSCs were used in combination to co-culture chondrocytes, the effect on genes was the most significant, while IL-1 β treatment overtly reversed the effects of SDMSC and ADMSCs alone or in combination on COL2A1, Aggrecan, SOX9 and MMP-13 expressions. That is, IL-1 β inhibited the expressions of COL2A1, Aggrecan and SOX9, but promoted the expression of MMP-13 (Figure 1A, $p < 0.001$). Inflammation could cause oxidative stress, and the reduction of ROS, a specific indicator of oxidative stress, led to the suppression of inflammatory signals (P-NF- κ B and IL-6).¹⁹ Therefore, we further examined ROS level in IL-1 β -induced cells to figure out the interaction between oxidative stress and inflammatory response in the progression of arthritis. Then, the ROS level in each group was detected by the ROS kit. The results mirrored that both SDMSCs and ADMSCs reduced IL-1 β -induced ROS level, and the effect of SDMSCs and ADMSCs in combination was much more obvious (Figure 1B, $p < 0.05$, $p < 0.01$, $p < 0.001$). The expressions of inflammatory cytokines (IL-6, TNF- α and IL-1 β) in each group were detected by corresponding ELISA kits, respectively. The results revealed that SDMSCs and ADMSCs in combination lessened the levels of IL-6, TNF- α and IL-1 β , and explicitly offset the effects of IL-1 β on promoting the levels of inflammatory cytokines. Moreover, the combination of SDMSCs and ADMSCs exerted a far more conspicuous effect on reducing the levels of inflammatory factors (Figure 1C–E, $p < 0.05$, $p < 0.01$, $p < 0.001$).

3.2 | Effects of SDMSCs and ADMSCs on joint diameter difference and pathological changes of femoral medial condyle articular cartilage in OA rats

The OA model rats were treated with SDMSCs and ADMSCs for 8 weeks, and the difference in knee joint diameter was evaluated. The results showed that compared with the sham group, the difference in knee joint diameter of the model group was significantly increased (Figure 2A, $p < 0.001$), indicating that the circumference of the knee joint on the surgical side was increased, and the OA model was successfully established. Compared with the OA group, the difference in the diameter of the knee joints in the rats was significantly reduced (Figure 2A, $p < 0.001$) in the OA + SDMSCs or OA + ADMSCs group, and the most obvious difference was observed in the OA + SDMSCs + ADMSCs group. Meanwhile, there was no marked difference between the OA + SDMSCs group and OA + ADMSCs group. These findings collectively indicated that SDMSCs and ADMSCs in combination might improve the knee joint diameter of OA rats.

Furthermore, HE staining and Safranin-O-fast green staining were employed to observe the pathological changes in cartilage tissue of medial femoral condyle of rats (Figure 2B). HE staining exhibited that in rats of the sham group, the articular bone tissue was

normal, and the chondrocytes were evenly distributed, with regular arrangement, clearly visible structure, and complete and smooth tidal line. By contrast, in the OA group, the knee joint of rats was rough, the thickness of the cartilage as well as the number of chondrocytes were clearly diminished, with obvious fibrosis. Besides, the SDMSCs group and ADMSCs group appeared apparently ameliorated cartilage injury, increased cartilage thickness, and reduced fissures, but the cartilage thickness and chondrocyte number were still less than the sham group. Additionally, the cartilage thickness, chondrocyte number, and surface regularity in OA + SDMSCs + ADMSCs group were equivalent to those in the sham group. Safranin-O staining unraveled that the staining intensity of knee joint cartilage in the OA group was low, accompanied with pale color, and lost chondrocytes, but the staining was deeper after injection of SDMSCs or ADMSCs, and the staining was dark red after combined application of SDMSCs and ADMSCs, suggesting that the formation of knee joint articular cartilage in rats was gradually facilitated, while the cartilage degradation was declined.

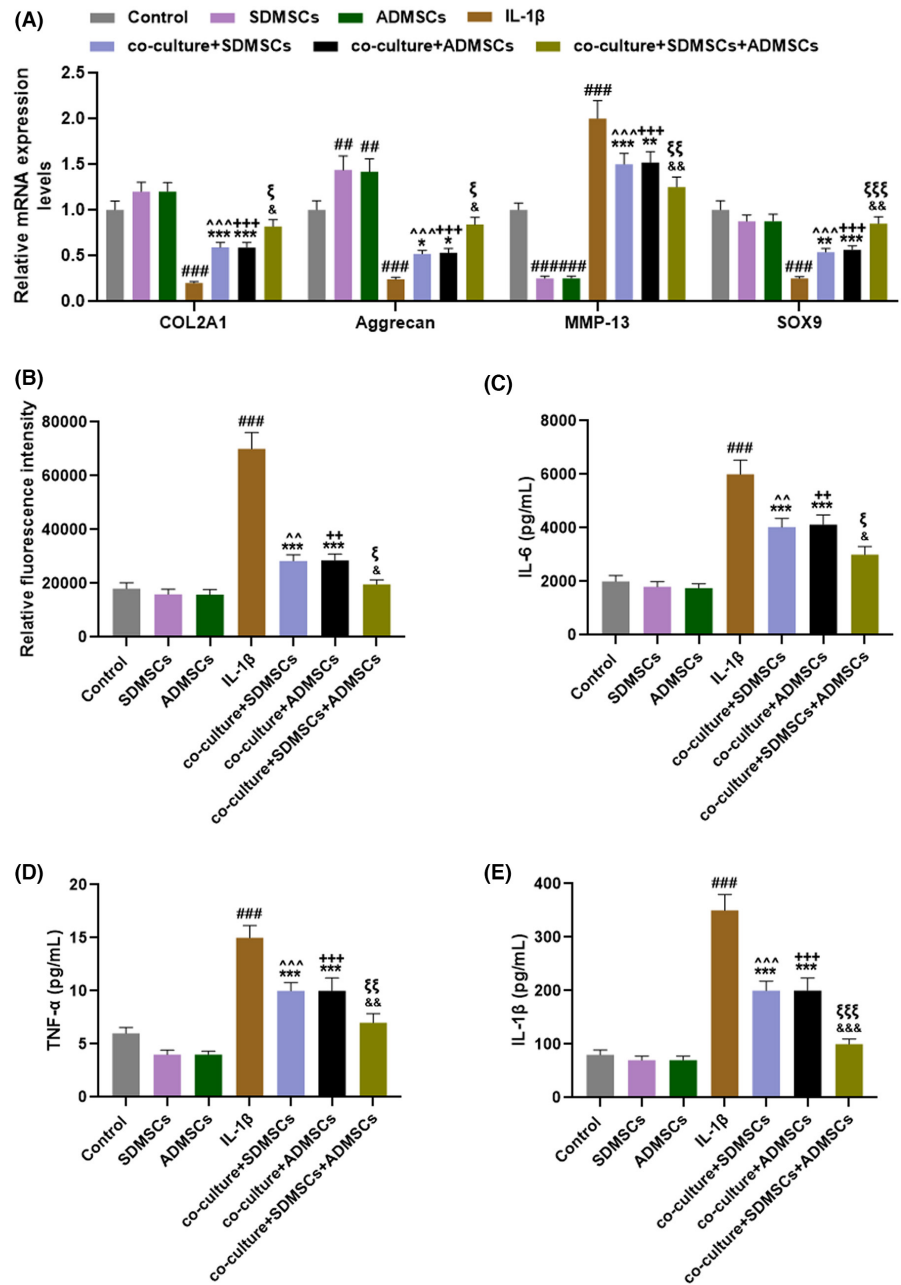
3.3 | Effects of SDMSCs and ADMSCs on the levels of inflammatory factors and ROS content in joint fluid of OA rats

ROS kit was utilized to detect the ROS level in cartilage tissues of rats in each group. The results showed that ROS level in articular fluid of rats in OA group was significantly increased, and SDMSCs or ADMSCs alone could markedly reduce ROS level, with no significant difference between the SDMSCs group and ADMSCs group. Compared with monotherapy, the level of ROS was decreased more explicitly after the combination of SDMSCs and ADMSCs (Figure 3A, $p < 0.05$, $p < 0.001$). Through the detection on levels of inflammatory factors in rat joint cavity fluid by ELISA, the levels of IL-6, TNF- α and IL-1 β were found to be dramatically boosted in OA rats ($p < 0.001$), while the injection of SDMSCs or ADMSCs alone could significantly reduce the levels of IL-6, TNF- α and IL-1 β ($p < 0.001$), with no marked difference between SDMSCs group and ADMSCs group. Moreover, as compared with the injection of SDMSCs or ADMSCs alone, the combination of SDMSCs and ADMSCs decreased the levels of IL-6, TNF- α and IL-1 β more overtly in the OA rats (Figure 3B–D, $p < 0.001$).

3.4 | Effects of SDMSCs and ADMSCs on chondrogenic markers and inflammatory factors in OA rats

To further investigate the effects of SDMSCs and ADMSCs on chondrogenicity in OA rats, we further examined the expressions of COL2A1, Aggrecan, MMP-13 and SOX9. The results uncovered that in OA group, MMP-13 expression in articular cartilage of rats was apparently elevated, and the expressions of COL2A1, Aggrecan and SOX9 were evidently reduced. However, complete opposite trends were shown in the SDMSCs and ADMSCs groups, that is, MMP-13

FIGURE 1 Effects of cell co-culture on chondrogenesis, oxidative stress and inflammatory factors. (A) QRT-PCR was used to detect the expressions of COL2A1, Aggrecan, SOX9 and MMP-13 in co-cultured cells (articular cartilage cells, SDMSCs and ADMSCs). (B) The ROS level of rats in each group was detected by reactive oxygen species (ROS) kit. (C–E) The expressions of inflammatory cytokines in each group were determined by IL-6-, TNF- α - and IL-1 β -associated ELISA kits, respectively. ($p < 0.05$, $**p < 0.01$, $***p < 0.001$, vs. IL-1 β ; $\hat{p} < 0.01$, $\hat{\hat{p}} < 0.001$, vs. SDMSCs; $++p < 0.01$, $+++p < 0.001$, vs. ADMSCs; $###p < 0.001$, vs. control; $\xi p < 0.05$, $\xi\xi p < 0.01$, $\xi\xi\xi p < 0.001$, vs. co-culture + SDMSCs; $\delta p < 0.05$, $\delta\delta p < 0.01$, $\delta\delta\delta p < 0.001$, vs. co-culture + ADMSCs)



level was conspicuously declined, whilst those of COL2A1, Aggrecan and SOX9 were overtly increased. Meanwhile, the most marked changes in the levels of aforementioned genes and proteins were observed in the OA + SDMSCs + ADMSCs group (Figure 4A,C,D, $p < 0.01$, $p < 0.001$). In addition, the inflammatory factors were detected by qRT-PCR and Western blotting in OA rat cartilage tissue, and the results were consistent with that of the determination on the chondrogenic marker expressions in joint fluid. Namely, the levels of IL-6, TNF- α and IL-1 β were significantly promoted in the OA rats, while the injection of SDMSCs or ADMSCs alone significantly reduced the levels of IL-6, TNF- α and IL-1 β . As compared with the injection of SDMSCs or ADMSCs alone, the combination of SDMSCs and ADMSCs lessened the levels of IL-6, TNF- α and IL-1 β more obviously in the OA rats (Figure 4B–D, $p < 0.05$, $p < 0.01$, $p < 0.001$).

4 | DISCUSSION

The proposal of stem cell therapy has provided new hope for OA with cartilage regeneration defect as the main pathological mechanism. However, this method has not achieved amazing results so far. The reason may be related to the inconsistent results caused by different cell types. Indira Prasad et al. proposed that BMMSCs combined with articular chondrocytes could improve OA, indicating that mixed cell therapy might be an effective strategy to improve OA cell therapy.¹⁵ Therefore, we proposed the method of combining ADMSCs with SDMSCs for the first time, which could utilize the easily obtained ADMSCs with good anti-inflammatory effect, as well as the regeneration, repair and self-renewal of cartilage of SDMSCs to achieve the purpose of synergistic treatment.

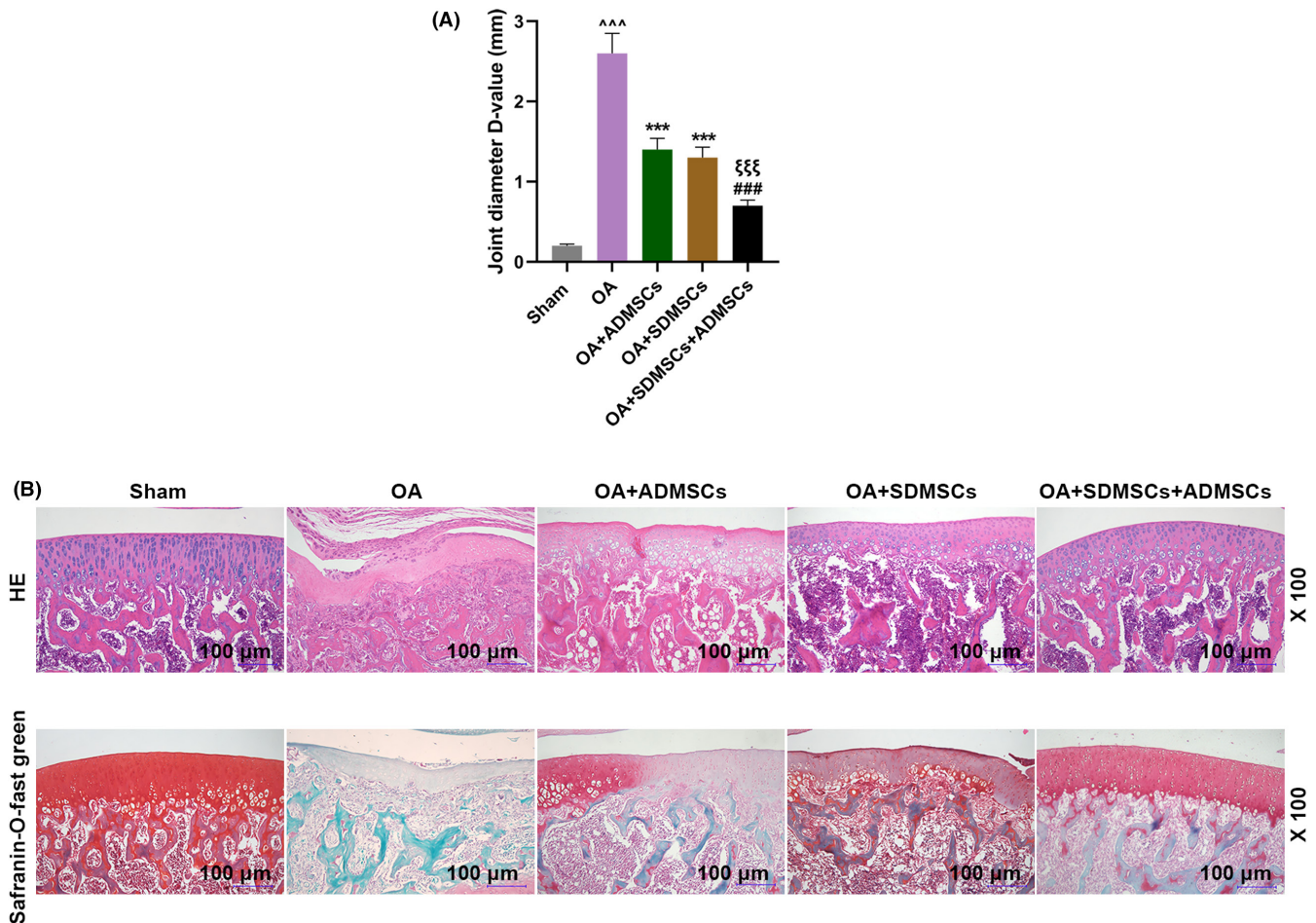


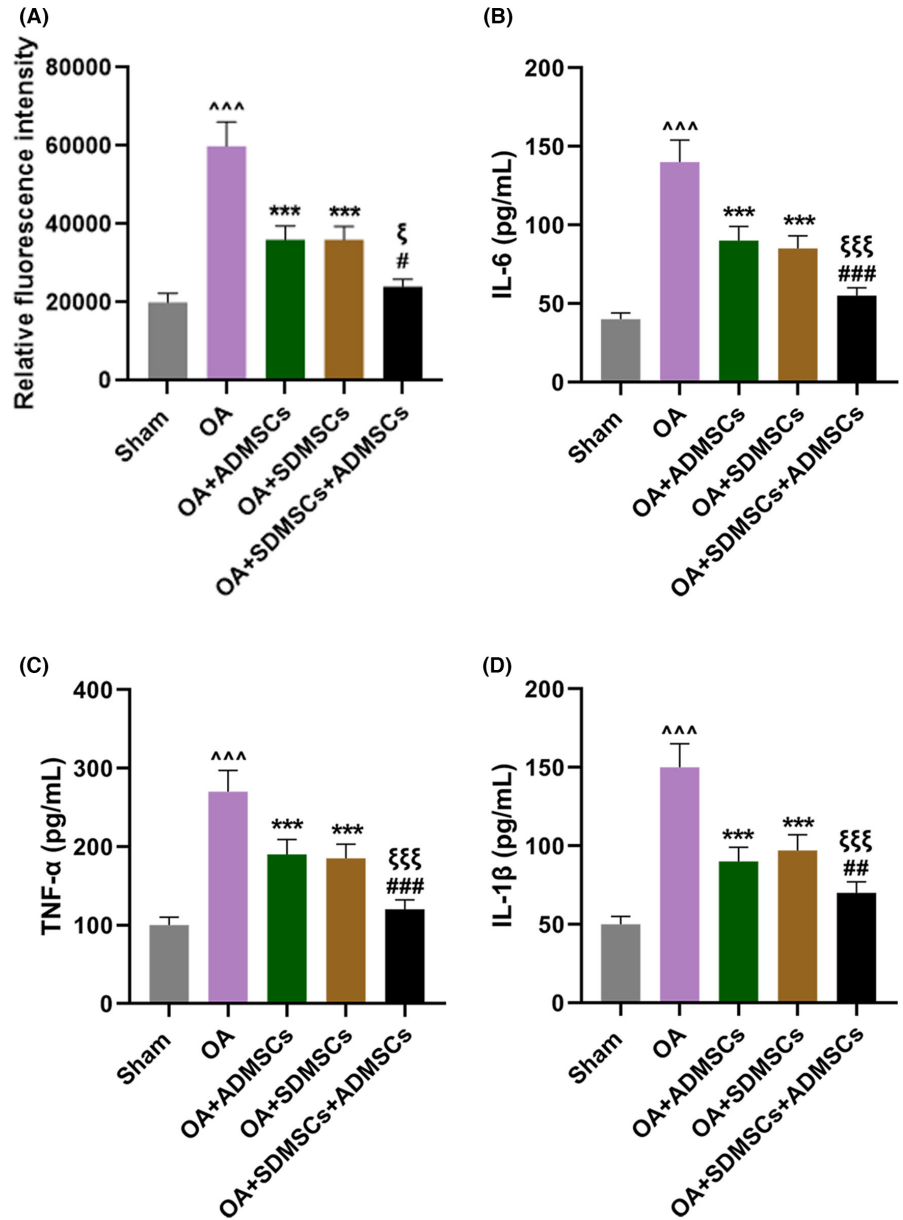
FIGURE 2 Effects of SDMSCs and ADMSCs on joint diameter difference and pathological changes of femoral medial condyle articular cartilage in OA rats. (A) Joint diameter difference of OA rats was detected. (B) HE staining and Safranin-O-fast green staining were used to observe the pathological changes in cartilage tissue of medial femoral condyle of rats. (** $p < 0.001$, vs. OA; ^^ $p < 0.001$, vs. Sham; ### $p < 0.001$, vs. OA + ADMSCs; ξξξ $p < 0.001$, vs. OA + SDMSCs)

Hulth surgery is a classic method for establishing OA models on animals, via which severe joint damage was induced due to joint instability through excision of the anterior cruciate ligament and unilateral meniscus. Persistent joint instability can cause cartilage and subchondral bone erosion degeneration, and these characteristics are highly similar to the pathological characteristics of human spontaneous OA. Thus, Hulth surgery is applied to create animal models of OA.²⁰ In this experiment, Hulth operation was used to successfully induce OA model in rats, and the therapeutic effect of intra-articular injection of ADMSCs and SDMSCs on OA rats' joint injury was evaluated. Several preclinical studies have demonstrated that either ADMSCs or SDMSCs is effective in reducing cartilage degeneration and joint inflammation in mice or rats with OA.^{17,21,22} Clinical experiments have also proved the good effect of ADMSCs in improving OA,²³ but the effect of SDMSCs on ameliorating OA has not been confirmed by clinical research. In addition, a clinical trial evaluated the efficacy of the combination of autologous BMMSCs and ADMSCs in the treatment of OA and found that such a combined treatment could improve knee joint function in all patients in synergy with BMMSCs-mediated cartilage regeneration and

ADMSCs-dependent immunosuppression.²⁴ However, SDMSCs have higher chondrogenic potential than BMMSCs,²⁵ so we chose to use SDMSCs instead of BMMSCs, and found that the combined injection of ADMSCs and SDMSCs has a better effect on reducing the joint damage of OA rats than injection of the cells alone.

This study has confirmed through experiments that the combination of two cells in the treatment of OA is more significant, which may be a new discovery in the exploration of the therapeutic mechanism of OA. Oxidative stress and inflammation in the pathogenesis of OA are interdependent. Many *in vitro* and *in vivo* studies have shown that IL-1 β stimulates the production of ROS in human and animal chondrocytes and promotes the apoptosis of chondrocytes.^{26,27} In addition, the increase in oxidative stress was positively correlated with the degradation of collagen, indicating the important role of ROS in the decomposition of cartilage matrix.²⁸ A previous study has reported that the combination of SDMSCs and apigenin (with significant antioxidant effect) can improve the symptoms of OA in rats by significantly reducing oxidative stress.²¹ Of note, ADMSCs have also been proved to have anti-oxidative stress and immunomodulatory effects.²⁹ In this study, we found that the combined treatment of

FIGURE 3 Effects of SDMSCs and ADMSCs on the levels of inflammatory factors and ROS content in joint fluid of OA rats. (A) ROS kit was utilized to detect the ROS level in cartilage tissues of rats in each group. (B) ELISA was applied to determine the IL-6 expression in joint fluid of OA rats treated with SDMSCs and ADMSCs. (C) ELISA was employed to measure the TNF- α expression in joint fluid of OA rats treated with SDMSCs and ADMSCs. (D) ELISA was exploited to test the IL-1 β expression in joint fluid of OA rats after treatment of SDMSCs and ADMSCs. (** $p < 0.001$, vs. OA; $^{^^}p < 0.001$, vs. Sham; $^{\#}p < 0.05$, $^{###}p < 0.001$, vs. OA + ADMSCs; $^{\xi}p < 0.05$, $^{\xi\xi}p < 0.01$, $^{\xi\xi\xi}p < 0.001$, vs. OA + SDMSCs)



ADMSCs and SDMSCs inhibited ROS production and inflammatory factor expression more effectively, indicating that the mechanism of improving OA by SDMSCs and ADMSCs may be related to reducing oxidative stress.

Transcription factor SOX9 is required for chondrogenic differentiation of MSCs,³⁰ which has a synergistic effect on chondrogenic differentiation and is a transcription factor necessary for the expressions of COL2A1, Aggrecan and Connexin.^{31,32} In addition, in the articular cartilage of patients with OA, the increase in MMP-13 content caused the destruction of the cartilage matrix network type-II collagen structure, resulting in articular cartilage defects.³³ A study compared the therapeutic effects of ADMSCs and SDMSCs on OA rats, and found that both of them could improve the progress of OA, but SDMSCs were better than ADMSCs in subchondral bone and cartilage mineralization.³⁴ However, in ADMSCs, MSCs derived from IPFP showed higher differentiation potential and expressed more

SOX-9, COL2A1, and Aggrecan than MSCs derived from subcutaneous fat.³⁵ Compared with other MSC sources, synovium is the closest to articular cartilage and can produce hyaline cartilage under benign conditions, so SDMSCs can improve OA by promoting ECM synthesis and increasing cartilage regeneration.³⁶ One study has reported that SDMSCs from patients with OA express MSCs markers twice as many cells as normal synovium, and moreover, they also have the ability to differentiate into chondrocytes *in vitro*.³⁷ In addition, there were no significant differences in the chondrogenic potential of synovial membranes from different sites.³⁸ Of note, chondrocytes can resist oxidative stress by producing antioxidant enzymes, while the suppressed production of ROS can improve the function of chondrocytes.³⁹ In this study, the combined treatment of ADMSCs and SDMSCs increased the expression of SOX9, suggesting that the combination of ADMSCs and SDMSCs may protect chondrocytes from cell dysfunction and cell death by reducing oxidative stress.

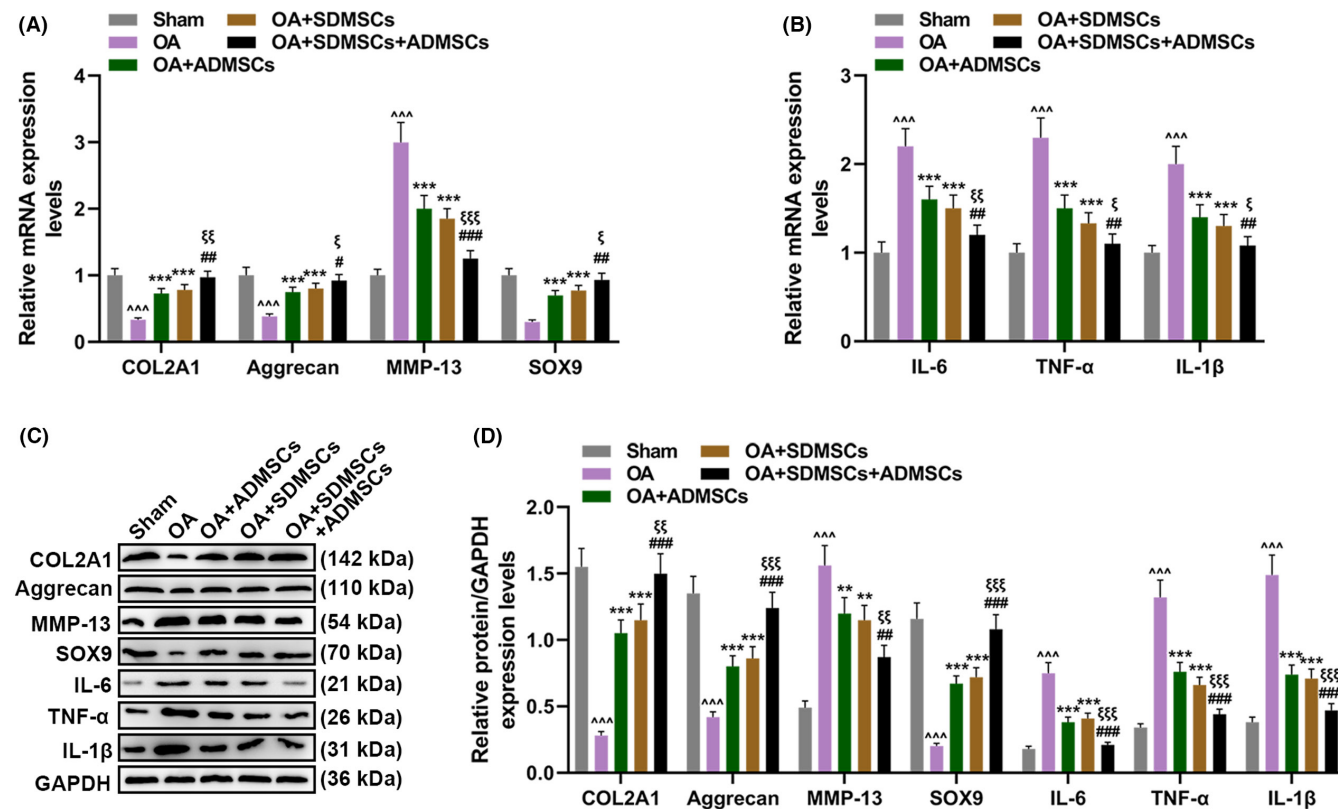


FIGURE 4 Effects of SDMSCs and ADMSCs on chondrogenic markers and inflammatory factors in OA rats. (A) QRT-PCR was used to examine the expressions of COL2A1, Aggrecan, MMP-13 and SOX9 in cartilage tissue of OA rats treated with SDMSCs and ADMSCs. (B) QRT-PCR was employed to examine the expressions of IL-6, TNF- α and IL-1 β in cartilage tissue of OA rats treated with SDMSCs and ADMSCs. (C–D) Western blotting was utilized to measure the expressions of COL2A1, Aggrecan, MMP-13, SOX9, IL-6, TNF- α and IL-1 β in cartilage tissue of OA rats treated with SDMSCs and ADMSCs. (***) $p < 0.001$, vs. OA; (^^) $p < 0.001$, vs. Sham; (#) $p < 0.05$, (##) $p < 0.01$, (###) $p < 0.001$, vs. OA + ADMSCs; (ξ) $p < 0.05$, ($\xi\xi$) $p < 0.01$, ($\xi\xi\xi$) $p < 0.001$, vs. OA + SDMSCs)

However, this study also has some shortcomings. For instance, this study did not use allogeneic transplantation but xenotransplantation, which could not reflect the advantage of MSCs in avoiding allogeneic rejection. In addition, although we suggested that ADMSCs combined with SDMSCs might improve OA by reducing oxidative stress and protecting chondrocytes, the specific mechanism still needed to be further clarified.

In summary, ADMSCs and SDMSCs improve OA rats by reducing chondrocyte ROS and inhibiting inflammatory response.

CONFLICT OF INTEREST

The authors declare no conflicts of interests.

AUTHOR CONTRIBUTIONS

Substantial contributions to conception and design: Xunzhi Liu, Yaqing Liu. Data acquisition, data analysis, and interpretation: Huabin He, Weiwei Xiang, Cheng He. Drafting the article or critically revising it for important intellectual content: Xunzhi Liu, Yaqing Liu. Final approval of the version to be published: Xunzhi Liu, Yaqing Liu, Huabin He, Weiwei Xiang, Cheng He. Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy

or integrity of the work are appropriately investigated and resolved: Xunzhi Liu, Yaqing Liu, Huabin He, Weiwei Xiang, Cheng He.

DATA AVAILABILITY STATEMENT

The analyzed data sets generated during the study are available from the corresponding author on reasonable request.

ORCID

Xunzhi Liu  <https://orcid.org/0000-0002-9933-6799>

REFERENCES

- Herrero-Beaumont G, Pérez-Baos S, Sánchez-Pernaute O, Roman-Blas JA, Lamuedra A, Largo R. Targeting chronic innate inflammatory pathways, the main road to prevention of osteoarthritis progression. *Biochem Pharmacol*. 2019;165:24-32. doi:10.1016/j.bcp.2019.02.030
- O'Connell NE, Smith KJ, Peterson MD, et al. Incidence of osteoarthritis, osteoporosis and inflammatory musculoskeletal diseases in adults with cerebral palsy: a population-based cohort study. *Bone*. 2019;125:30-35. doi:10.1016/j.bone.2019.05.007
- Guedes V, Castro JP, Brito I. Topical capsaicin for pain in osteoarthritis: a literature review. *Reumatol Clin*. 2018;14(1):40-45. doi:10.1016/j.reuma.2016.07.008

4. Lu CH, Chung CH, Lee CH, et al. Combination COX-2 inhibitor and metformin attenuate rate of joint replacement in osteoarthritis with diabetes: a nationwide, retrospective, matched-cohort study in Taiwan. *PLoS One*. 2018;13(1):e0191242. doi:10.1371/journal.pone.0191242
5. van Walsem A, Pandhi S, Nixon RM, Guyot P, Karabis A, Moore RA. Relative benefit-risk comparing diclofenac to other traditional non-steroidal anti-inflammatory drugs and cyclooxygenase-2 inhibitors in patients with osteoarthritis or rheumatoid arthritis: a network meta-analysis. *Arthritis Res Ther*. 2015;17(1):66. doi:10.1186/s13075-015-0554-0
6. Kim CH, Lim CY, Lee JH, Kim KC, Ahn JY, Lee EJ. Human embryonic stem cells-derived mesenchymal stem cells reduce the symptom of psoriasis in imiquimod-induced skin model. *Tissue Eng Regen Med*. 2019;16(1):93-102. doi:10.1007/s13770-018-0165-3
7. Richardson SM, Kalamegam G, Pushparaj PN, et al. Mesenchymal stem cells in regenerative medicine: focus on articular cartilage and intervertebral disc regeneration. *Methods*. 2016;99:69-80. doi:10.1016/j.ymeth.2015.09.015
8. McKinney JM, Doan TN, Wang L, et al. Therapeutic efficacy of intra-articular delivery of encapsulated human mesenchymal stem cells on early stage osteoarthritis. *Eur Cell Mater*. 2019;37:42-59. doi:10.22203/eCM.v037a04
9. Zellner J, Pattappa G, Koch M, et al. Autologous mesenchymal stem cells or meniscal cells: what is the best cell source for regenerative meniscus treatment in an early osteoarthritis situation? *Stem Cell Res Ther*. 2017;8(1):225. doi:10.1186/s13287-017-0678-z
10. Jezierska-Woźniak K, Nosarzewska D, Tutas A, Mikołajczyk A, Okliński M, Jurkowski MK. Use of adipose tissue as a source of mesenchymal stem cells. *Postepy Hig Med Dosw (Online)*. 2010;64:326-332.
11. Harrell CR, Markovic BS, Fellabaum C, Arsenijevic A, Volarevic V. Mesenchymal stem cell-based therapy of osteoarthritis: current knowledge and future perspectives. *Biomed Pharmacother*. 2019;109:2318-2326. doi:10.1016/j.biopha.2018.11.099
12. Reisbig NA, Hussein HA, Pinnell E, Bertone AL. Evaluation of equine synovial-derived extracellular matrix scaffolds seeded with equine synovial-derived mesenchymal stem cells. *Am J Vet Res*. 2018;79(1):124-133. doi:10.2460/ajvr.79.1.124
13. Sekiya I, Muneta T, Horie M, Koga H. Arthroscopic transplantation of synovial stem cells improves clinical outcomes in knees with cartilage defects. *Clin Orthop Relat Res*. 2015;473(7):2316-2326. doi:10.1007/s11999-015-4324-8
14. Ntege EH, Sunami H, Shimizu Y. Advances in regenerative therapy: a review of the literature and future directions. *Regen Ther*. 2020;14:136-153. doi:10.1016/j.reth.2020.01.004
15. Prasadam I, Akuien A, Friis TE, et al. Mixed cell therapy of bone marrow-derived mesenchymal stem cells and articular cartilage chondrocytes ameliorates osteoarthritis development. *Lab Invest*. 2018;98(1):106-116. doi:10.1038/labinvest.2017.117
16. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods*. 2001;25(4):402-408. doi:10.1006/meth.2001.1262
17. Zhou J, Wang Y, Liu Y, Zeng H, Xu H, Lian F. Adipose derived mesenchymal stem cells alleviated osteoarthritis and chondrocyte apoptosis through autophagy inducing. *J Cell Biochem*. 2018;120(2):2198-2212. doi:10.1002/jcb.27530
18. Zwickl H, Niculescu-Morzea E, Halbwirth F, et al. Correlation analysis of SOX9, -5, and -6 as well as COL2A1 and aggrecan gene expression of collagen I implant-derived and osteoarthritic chondrocytes. *Cartilage*. 2016;7(2):185-192. doi:10.1177/1947603515615388
19. Lin HJ, Ramesh S, Chang YM, et al. D-galactose-induced toxicity associated senescence mitigated by alpinate oxyphyllae fructus fortified adipose-derived mesenchymal stem cells. *Environ Toxicol*. 2020;36(1):86-94. doi:10.1002/tox.23014
20. Hulth A, Lindberg L, Telhag H. Experimental osteoarthritis in rabbits. Preliminary report. *Acta Orthop Scand*. 1970;41(5):522-530. doi:10.3109/17453677008991540
21. Estakhri F, Reza Panjehshahin M, Tanideh N, Gheisari R, Azarpira N, Gholijani N. Efficacy of combination therapy with apigenin and synovial membrane-derived mesenchymal stem cells on knee joint osteoarthritis in a rat model. *Iran J Med Sci*. 2021;46(5):383-394. doi:10.30476/ijms.2020.83686.1301
22. Mak J, Jablonski CL, Leonard CA, et al. Intra-articular injection of synovial mesenchymal stem cells improves cartilage repair in a mouse injury model. *Sci Rep*. 2016;6:23076. doi:10.1038/srep23076
23. Lee WS, Kim HJ, Kim KI, Kim GB, Jin W. Intra-articular injection of autologous adipose tissue-derived mesenchymal stem cells for the treatment of knee osteoarthritis: a phase IIb, randomized, placebo-controlled clinical trial. *Stem Cells Transl Med*. 2019;8(6):504-511. doi:10.1002/sctm.18-0122
24. Kim JD, Lee GW, Jung GH, et al. Clinical outcome of autologous bone marrow aspirates concentrate (BMAC) injection in degenerative arthritis of the knee. *Eur J Orthop Surg Traumatol*. 2014;24(8):1505-1511. doi:10.1007/s00590-013-1393-9
25. Sasaki A, Mizuno M, Ozeki N, et al. Canine mesenchymal stem cells from synovium have a higher chondrogenic potential than those from infrapatellar fat pad, adipose tissue, and bone marrow. *PLoS One*. 2018;13(8):e0202922. doi:10.1371/journal.pone.0202922
26. Ansari MY, Ahmad N, Haqqi TM. Oxidative stress and inflammation in osteoarthritis pathogenesis: role of polyphenols. *Biomed Pharmacother*. 2020;129:110452. doi:10.1016/j.biopha.2020.110452
27. Ansari MY, Khan NM, Ahmad I, Haqqi TM. Parkin clearance of dysfunctional mitochondria regulates ROS levels and increases survival of human chondrocytes. *Osteoarthritis Cartilage*. 2018;26(8):1087-1097. doi:10.1016/j.joca.2017.07.020
28. Bolduc JA, Collins JA, Loeser RF. Reactive oxygen species, aging and articular cartilage homeostasis. *Free Radic Biol Med*. 2019;132:73-82. doi:10.1016/j.freeradbiomed.2018.08.038
29. Gao Y, Huang X, Lin H, et al. Adipose mesenchymal stem cell-derived antioxidative extracellular vesicles exhibit anti-oxidative stress and immunomodulatory effects under PM(2.5) exposure. *Toxicology*. 2021;447:152627. doi:10.1016/j.tox.2020.152627
30. Sadatsuki R, Kaneko H, Kinoshita M, et al. Perlecan is required for the chondrogenic differentiation of synovial mesenchymal cells through regulation of Sox9 gene expression. *J Orthop Res*. 2017;35(4):837-846. doi:10.1002/jor.23318
31. Honarpardaz A, Irani S, Pezeshki-Modaress M, Zandi M, Sadeghi A. Enhanced chondrogenic differentiation of bone marrow mesenchymal stem cells on gelatin/glycosaminoglycan electrospun nanofibers with different amount of glycosaminoglycan. *J Biomed Mater Res A*. 2019;107(1):38-48. doi:10.1002/jbm.a.36501
32. Kurakazu I, Akasaki Y, Hayashida M, et al. FOXO1 transcription factor regulates chondrogenic differentiation through transforming growth factor β 1 signaling. *J Biol Chem*. 2019;294(46):17555-17569. doi:10.1074/jbc.RA119.009409
33. Bo W, Zhou J, Wang K. Sodium butyrate abolishes the degradation of type II collagen in human chondrocytes. *Biomed Pharmacother*. 2018;102:1099-1104. doi:10.1016/j.biopha.2018.03.062
34. Zare R, Tanideh N, Nikahval B, et al. Are stem cells derived from synovium and fat pad able to treat induced knee osteoarthritis in rats? *Int J Rheumatol*. 2020;2020:9610261. doi:10.1155/2020/9610261
35. Vahedi P, Moghaddamshahabi R, Webster TJ, et al. The use of infrapatellar fat pad-derived mesenchymal stem cells in articular

- cartilage regeneration: a review. *Int J Mol Sci.* 2021;22(17):9215. doi:[10.3390/ijms22179215](https://doi.org/10.3390/ijms22179215)
36. Fellows CR, Matta C, Zakany R, Khan IM, Mobasher A. Adipose, bone marrow and synovial joint-derived mesenchymal stem cells for cartilage repair. *Front Genet.* 2016;7:213. doi:[10.3389/fgene.2016.00213](https://doi.org/10.3389/fgene.2016.00213)
37. Hermida-Gómez T, Fuentes-Boquete I, Gimeno-Longas MJ, et al. Quantification of cells expressing mesenchymal stem cell markers in healthy and osteoarthritic synovial membranes. *J Rheumatol.* 2011;38(2):339-349. doi:[10.3899/jrheum.100614](https://doi.org/10.3899/jrheum.100614)
38. Mochizuki T, Muneta T, Sakaguchi Y, et al. Higher chondrogenic potential of fibrous synovium- and adipose synovium-derived cells compared with subcutaneous fat-derived cells: distinguishing properties of mesenchymal stem cells in humans. *Arthritis Rheum.* 2006;54(3):843-853. doi:[10.1002/art.21651](https://doi.org/10.1002/art.21651)
39. Lepetos P, Papavassiliou AG. ROS/oxidative stress signaling in osteoarthritis. *Biochim Biophys Acta.* 2016;1862(4):576-591. doi:[10.1016/j.bbadis.2016.01.003](https://doi.org/10.1016/j.bbadis.2016.01.003)

How to cite this article: Liu X, Liu Y, He H, Xiang W, He C. Human adipose and synovial mesenchymal stem cells improve osteoarthritis in rats by reducing chondrocyte reactive oxygen species and inhibiting inflammatory response. *J Clin Lab Anal.* 2022;36:e24353. doi:[10.1002/jcla.24353](https://doi.org/10.1002/jcla.24353)