Joint Distraction Combined with Mesenchymal Stem Cell Intra-articular Injection Attenuates Osteoarthritis

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Abstract

Background: Conservative treatments of osteoarthritis (OA) are limited to symptom relief and novel methods to attenuate OA progression are lacking.

Objective: In this study, we investigated the effectiveness of knee joint distraction (KJD) combined with mesenchymal stem cells (MSCs) intra-articular injection (KJD+MSCs) in OA rat model.

Methods: OA rat model was established by anterior cruciate ligament transection plus medial meniscus resection in right knee in SD rat. The KJD+MSCs treatment started 3 weeks after the OA surgery. There were two other groups as knee joint distraction only (KJD) and no treat (OA). Three weeks after the treatment, distraction external fixators were removed and rats were kept for further 3 weeks. The rats were then terminated, samples were subject to micro-CT and histology examinations to evaluate the changes of the articular cartilage tissues, subchondral bone and the secondary inflammation.

Results: Safranin-O/fast green staining showed that articular cartilage injury was most obvious in the OA group than that in the KJD group and the least in the KJD+MSCs group. Immunohistochemistry examinations showed that the KJD+MSCs group had the lowest percentage of MMP13 or ColX positive chondrocytes comparing to other groups. Micro-CT data indicated that the abnormal change in the subchondral region of the tibia in the KJD+MSCs group was significantly less than that in the KJD group or OA group. Finally, immunohistochemistry result showed that the knee joint in the KJD+MSCs group had the least number of CD68-positive cells among all the groups.

Conclusions: Joint distraction combined with mesenchymal stem cells injection alleviated cartilage degradation, reduced irregular ossification of subchondral bone and secondary inflammation, suggesting it could be a new method to halt the OA progression.

Introduction

Osteoarthritis (OA) is a common degenerative disease of joints. Its pathological features are mainly cartilage degenerative disease, irregular subchondral ossification and secondary inflammation of synovial membrane[1–3]. The clinical treatment of OA is very limited. Drug therapy can only relieve the pain of the knee joint, but not alleviate the progress of the disease. Many patients must undergo total arthroplasty to alleviate stiffness and improve the function of joint movement in the later stages of disease. Epidemiological studies show that about 44% of patients receiving total arthroplasty are younger than 65 years old[4], which means they may have to face joint revision surgery in their later years. Therefore, new therapies are urgently needed to slow the progress of OA.

Abnormal mechanical loading plays an important role in OA progression[5–8]. Joint distraction is a novel therapy that can modulate this overloading and enable intrinsic joint tissue regeneration supposedly by correcting the proper biochemical and biomechanical joint homeostasis[8, 9]. Joint distraction is a surgical procedure in which the two bony ends of a joint are gradually separated to a certain extent and

for a certain period of time by use of an external fixation frame. In the first report in 1978, Judet and his team pulled an ankle joint for a large cartilage defect through a movable hinged external fixator, and the results showed that the movable joint distraction treatment made the defect in the load-bearing area repair satisfactorily[10]. Subsequently, the treatment of OA by joint distraction received more attention. Following on from series of animal and clinical experiments on joint distraction, satisfactory results have shown that joint distraction can promote cartilage repair, alleviate pain in patients with OA, and improve quality of life [7, 11]. In a clinical study, Professor Lafeber's team evaluated the 9-year treatment outcome of knee joint distraction (KJD) and found that KJD promoted long-lasting clinical and structural improvement[11]. In our previous study, we found that KJD can attenuate OA progression by reducing cartilage damage, subchondral bone abnormalities, and secondary inflammation in a rat OA model[7]. Unfortunately, even though the experimental treatment of OA by joint distraction has made great progress, there are still some limitations. First, almost all researchers focus on the alleviation of cartilage degradation in OA by joint distraction, while there are few reports on the other two important pathological manifestations of OA (subchondral osteosclerosis and secondary inflammation). This underlies deficiencies in understanding the mechanism of the treatment of OA by joint distraction. Second, in a clinical study, the treatment of OA by joint distraction alone takes a relatively long period of time, usually 2–3 months, which affects the quality of daily life of patients. Also, there are complications such as nail tract infection[12, 13], which can increase the risk of affecting the success of joint replacement in the future. To shorten the treatment time of joint distraction and give patients a good treatment experience, a better treatment needs to be found.

Mesenchymal stem cells (MSCs) are a kind of stem cell with a wide range of sources, easy to collect and with multi-differentiation potential. They have long been considered to be an ideal source of cells for stem cell therapy[14–17]. In animal or clinical experiments, there have been many reports of MSCs intra-articular injection for the treatment of OA. Overall, these reports indicated that the use of MSCs in the treatment or prevention of OA can alleviate cartilage degradation and subchondral sclerosis[7, 18–20]. For example, in the articles published by our research group in 2017, MSCs pre-treated by chondrogenesis induction and differentiation and then reverse-differentiated into stem cells had a better therapeutic effect than without pre-treated MSCs or no MSCs treatment in a rat OA model and the underlining mechanism is mainly through the epigenetic modification[19, 20]. In clinical research, Wakitani et al.[21] first reported the application of MSCs mixed with type I collagen hydrogel transplantation into the cartilage defect in OA patients. Arthroscopic results showed that 24 weeks later, the original cartilage defect had been repaired by white tissue and the morphology was similar to hyaline cartilage. The biopsy section analysis also confirmed that hyaline cartilage regeneration did exist in the transplantation site. In another study, published by our research team, we found that MSC injection treatment has a potential therapeutic effect for wrist OA, as shown by numerical improvement in performance and pain scores[22]. From these reports of preclinical and clinical studies, it has been shown that MSC injection can have a positive effect as an OA therapy. However, some researchers believe that MSC injection can only be used in the early stage of OA. For the advanced stage, the severe subchondral sclerosis of the joints and increasing secondary inflammation are a hostile micro-environment to MSCs, which would hinder their therapeutic

effect[23]. Therefore, finding a combination therapy which can modulate the micro-environment of the joint will greatly improve the treatment of OA with MSC transplantation. Joint distraction may allow that modulation.

In this study, we explore the feasibility of knee joint distraction combined with mesenchymal stem cell injection (KJD + MSCs) in the treatment of OA in a rat model. We focus on the effects on this treatment in cartilage degeneration, irregular subchondral bone remodeling and secondary inflammation, which are known as the characteristic changes of OA.

Materials And Methods

Isolation and cultivation of MSCs

MSCs were isolated and cultivated from transgenic Sprague Dawley (SD) rats with green fluorescent protein (GFP) in Professor Gang Li's Laboratory at the Prince of Wales Hospital, Chinese University of Hong Kong. First, one week old transgenic SD rats were killed and complete femurs were placed in DMEM culture medium with 10% fetal bovine serum (FBS). Second, marrow was removed from the marrow cavity with a one milliliter syringe to get as much bone marrow as possible into the cell culture dish. Third, the cell suspension was put into an incubator. The culture medium was exchanged to remove non-adherent cells after 48 hours. When bone marrow cells reached about 80% confluence, the cells were digested with 0.25% trypsin (Amersco, Ohio, USA) containing 0.02% EDTA and passaged at 1:3. MSCs can be used in subsequent experiments in the 3rd to 6th generations.

Animals

All SD rats were 16 weeks old and weighed 450–500 g. All animal experiments were approved by the Animal Ethics Committee of Jinan University (Ethics Reference No.: 20180824-04). Only the right knee of the rats received the surgery. Each rat was injected with a solution of 0.2% (vol/vol) xylazine and 1% (vol/vol) ketamine in PBS for anesthesia. The right hind leg was disinfected with 70% alcohol after removing the hair and then was exposed through a medial parapatellar approach. The patella was dislocated to the lateral side and the knee joint was fixed in the full flexion position. Then the anterior cruciate ligament was transected and the medial meniscus was resected using micro-scissors (ACLT + MMx). After the surgery, the incisions were sutured in turn.

The rats were nursed normally for three weeks after surgery without any restriction on activity. Previous studies have shown that rats have persistent pathological changes of post-traumatic OA by this time[24]. The rats were randomly divided into three groups (n = 5 each): an OA group, a knee joint distraction group (KJD) and a knee joint distraction combined with mesenchymal stem cells (MSCs) injection group (KJD + MSCs). The rats in the OA group were treated as controls. In the KJD group, the rats were fitted with an external fixator and treated with joint distraction for three weeks. The KJD + MSCs group rats received joint distraction therapy, and then MSCs were injected into the joint cavity (100 μl, 0.5×10^6 cells) three days after joint distraction. The cells were mixed with clinical sodium hyaluronic acid (HA) (Biochemical

Industry Corporation; Imported Drug Registration Certificate No. H20140533). In the KJD, HA without cells, was injected as a control. In order to study whether this therapy has a relatively long-term therapeutic effect, we did not immediately sacrifice the rats after treatment, but dismantled the external fixator three weeks after the joint distraction treatment, without restricting the activity of rats, and followed up for another three weeks. After that, the rats were killed with an excessive dose of anesthetics, and the samples were taken for further analysis (Fig. 1).

Joint distraction procedure

We designed a specific external fixator for this study. This external fixator consists of three nails (1.2 mm in diameter) that are surgically drilled into the medial side of the knee joint. With the customized three-point positioner, we fixed the uppermost nail to the medial epicondyle of the femur, and the other two nails to the upper segment of the tibia. To ensure joint mobility during distraction, we added a customized cannula (1.3 mm in diameter) to the nail of the medial epicondyle of the femur. Finally, the customized external fixator was fixed to the three nails. In rats receiving joint distraction, the joint space was stretched by one mm. This distance was measured by X-ray as a reference for the normal joint space of the control side[7]. The maximum flexion and extension angle of the knee joint was observed after the operation. We used X-ray to detect the success of joint distraction before and after surgery. All animals were allowed to move freely without restriction post-operatively (Fig. 1).

Digital radiographs

Joint space width of the rat right knee was measured using the digital X-ray (MX-20, Faxitron X-Ray Corp., Wheeling, IL, US) with an exposure time of 6000 ms and a voltage of 32 kV.

Micro-CT analysis

Changes and quantitative analysis of subchondral bone microstructures in rats were detected by high-energy micro-CT (UCT40, Scanco Medical, Basserdorf, Switzerland). At the end of the study, the knee joints of the rats were separated with all corresponding soft tissues removed at the same time, fixed in 10% formalin for 24 hours, and then examined by μCT. A three-dimensional reconstruction (3D) image of mineralized tissue was made of the subchondral bone area of the tibia on one side of the rat. The domain value was 160 mg hydroxyapatite/cm^3) and a Gaussian filter (sigma = 0.8, support = 2) was used to suppress noise. Sagittal images of the tibial subchondral bone were used to perform 3D histomorphometric analysis. We defined the region of interest to cover the whole subchondral bone medial compartment and used a total of 100 consecutive images from the medial tibial plateau for 3D reconstruction and analysis.

Morphological and Immunohistochemical analysis

After removing the soft tissue from the knee joint of rats, the samples were fixed in 10% formalin for 48 hours, then decalcified in 10% EDTA for 21 days, and finally embedded in paraffin. Sagittal sections 5 μm thick were performed of the whole right knee. The section was mounted on a slide and stained with safranin-O/fast green.

Immunohistochemical and immunofluorescence staining used standard protocols. We incubated sections with primary antibodies of rabbit anti-rat MMP13 (1:50, ab3208; Abcam), collagen X (1:80, ab58632; Abcam) and CD68 (1:100, BA3638; Boster) overnight at 4 °C. For immunohistochemical staining, the slide was then incubated with HRP-conjugated goat anti-rabbit secondary antibody (Cell signal technology, 1:100, 7047) for 1 h at room temperature. Then a horseradish peroxidase-streptavidin detection system (Dako) was used to detect immunoactivity, followed by counterstaining with hematoxylin (Sigma). For Immunofluorescence staining, Cy3-conjugated goat anti-rabbit IgG (Jackson Immuno Research) secondary antibodies were used. Cell nuclei were counterstained with DAPI. Photo micrographs of selected areas were taken (Leica DMRB).

Statistical analysis

In accordance with the ARRIVE guidelines, we have reported measures of precision, confidence, and sample size to provide an indication of significance. All statistical analyses were performed using SPSS15.0 software. The data were analyzed via one-way ANOVA. Assumptions of the ANOVA were assessed using the Shapiro-Wilk test of normality and Levene's test for homogeneity of variance. The result of the Levene's test was used to determine the post hoc testing strategy. If not significant, the LSD-t post hoc test was employed. If Levene's test was significant, the ANOVA was followed by a Dunnett's T3 post hoc test for unequal variance. Data are reported as mean ± standard deviation, and values of $p < 0.05$ were considered significant. The graphs were generated in GraphPad Prism 6 (GraphPad Software, San Diego, CA, USA).

Results

Joint distraction combined with mesenchymal stem cell injection can alleviate cartilage degradation

X-rays of the knee joint indicated that KJD significantly enlarged the knee joint space in the distraction group compared with that in the OA group (**Fig. 2a**). Safranin-O/fast green staining showed that cartilage injury was most obvious in the OA group compared to the other two groups. KJD + MSCs had the smallest degree of cartilage damage among the three groups (Fig. 2b). More importantly, the number of MMP13 (an enzyme associated with the cartilage degradation) positive chondrocytes was significantly lower in the KJD + MSCs group than in the KJD or OA groups. Similarly, the number of Col X (a marker of the cartilage hypertrophy) positive chondrocytes in the KJD + MSCs group was significantly less than that in the KJD and OA groups (Fig. 3). These results suggest that joint distraction combined with mesenchymal stem cell injection can alleviate cartilage degradation and limit OA progression, and the therapeutic effect can last longer than joint distraction alone.

Joint distraction combined with mesenchymal stem cell injection can reduce irregular ossification of subchondral bone

The microstructure of the tibial subchondral bone in rats was demonstrated using μCT 3D reconstruction images (Fig. 4a). Bone mineral density (BMD) in the KJD + MSCs injection group (319.5 ± 87.97 mg/cm^3, n = 5) was significantly lower than that in the KJD or OA groups (411.7 ± 72.42 mg/cm^3, 467 ± 101.9 mg/cm^3, respectively; n = 5, p = 0.002) (Fig. 4b). The bone volume/total volume (BV/TV) in the KJD + MSCs injection group (0.311 ± 0.1117, n = 5) was significantly less compared with the KJD group (0.4849 ± 0.1042, n = 5) and the OA group (0.5563 ± 0.1386, n = 5, p = 0.0147) (Fig. 4c). Trabecular thickness (Tb.Th.) in the OA group (0.2372 ± 0.039 mm, n = 5) and the KJD group (0.1954 ± 0.034 mm, n = 5) were significantly greater than in the KJD + MSCs injection group (0.1698 ± 0.01598 mm, n = 5, p = 0.0268) (Fig. 4d). Moreover, trabecular bone space (Tb.Sp.) (Fig. 4e) was significantly lower in the OA group (0.2356 ± 0.061, n = 5) and the KJD group (0.2954 ± 0.034, n = 5) compared to the KJD + MSCs group (0.3819 ± 0.091, n = 5, p = 0.007). Finally, the structure model index (SMI) was significantly lower in the OA group (-2.438 ± 3.14, n = 5) and the KJD group (-0.6593 ± 1.59, n = 5), compared with the KJD + MSCs injection group (1.521 ± 1.41, n = 5, p = 0.036) (Fig. 4f).

Joint distraction combined with mesenchymal stem cells injection can reduce the secondary inflammation

To investigate whether this treatment would attenuate the secondary inflammation of the OA joint, CD68 (a marker of macrophages) positive cells were detected in the knee joint space of the affected joint in the different groups. Many cells infiltrated into the knee joint space in all three groups. We used immunohistochemical staining to analyze the number of CD68 positive cells in the knee joint space to measure the level of secondary inflammation. Several CD68 positive cells were found in the joint space in all three groups. Immunohistochemical staining showed that the level of secondary inflammation in the OA group was more severe than in the KJD and KJD + MSCs groups (Fig. 5). The fewest CD68 positive cells in the joint space were found in the KJD + MSCs group, indicating that KJD + MSCs treatment has excellent therapeutic effects on reducing secondary inflammation in the OA joint (Fig. 5).

Discussion

In this study, we used an ACLT + MMx OA model to evaluate the effect of KJD + MSCs intra-articular injection on OA. We found that KJD + MSCs injection can reduce secondary inflammation, cartilage degradation and irregular ossification of the subchondral bone effectively. Although in this experiment, KJD alone showed improvement over the non-treated OA group, combination therapy was significantly better than both. The mechanism is hypothesized to be due to joint distraction relieving aberrant mechanical stress and MSCs regulating the inflammation, allowing intrinsic tissue repair of the joint. Therefore, it is indicated that KJD combined with MSCs injection can achieve a mutually reinforcing therapeutic effect in OA treatment.

The therapy of young OA patients is a worldwide challenge. Younger patients have a high demand for joint functional activities, which will accelerate the wear of artificial joints. Second, most joint replacement patients face the problem of revision surgery due to the limitation of the service life of

current joint prostheses. Therefore, clinical and basic researchers are trying to find alternative treatments for this group of patients. Since the first use of joint distraction in the treatment of arthritis, it has been considered an alternative treatment for younger patients with OA[25], and clinical and animal experiments have shown the advantages of this treatment approach[26]. For example, Yang Xu et al.[27] used joint distraction to treat severe traumatic ankle arthritis in patients with an average age of 30.3 ± 14.3 years. After treatment, the joint pain of the patients was relieved, and the joint space increased by three mm after one year. In our previous study of KJD in the rat OA model, we observed that the cartilage defect of the treated group was smaller than that of the control group, and the inflammatory factors and subchondral bone mineral density also were better than those of the control group. Therefore, we inferred that joint distraction could decelerate secondary inflammation, cartilage degeneration and subchondral sclerosis[7]. However, in our previous study, we did not have a follow-up period, so we did not know how long the therapeutic effect lasted after joint distraction. To answer this question, in this study we defined a three week follow-up period after KJD treatment. Our results show that KJD + MSCs has a better therapeutic effect than KJD only or no treatment, as evidenced by the level of cartilage damage (Figs. 2–3), abnormal subchondral bone remodeling (Fig. 4) and secondary inflammation (Fig. 5). Our results are consistent with other research using KJD that this therapy can delay OA progression. We also found that the therapeutic effect is better when combined with MSCs, indicating that MSCs play an important role in regulating the micro-environment of the OA joint (Figs. 2–5).

MSCs are a kind of adult stem cell with multiple differentiation potential that can be isolated from various tissues such as bone marrow, adipose tissue, and synovium. MSC treatment of OA has been widely used in animal and clinical experiments. These experiments have demonstrated safety and are associated with cartilage regeneration, pain relief, and knee joint function improvement. Most of the experiments showed satisfactory results. According to previous experiments, it can be concluded that MSCs play a therapeutic role mainly through directional differentiation, regulation of immunity, and anti-inflammatory and exocrine effects[28–31]. For example, in a clinical study, published by our research team, we found that MSC injection treatment has a potential therapeutic effect for wrist OA, as shown by numerical improvement in performance and pain scores[22]. In animal studies published by our research group, we found that MSCs that were pre-treated by chondrogenesis induction and differentiation and then reverse-differentiated into stem cells, had better therapeutic effects by relieving cartilage damage and subchondral sclerosis than in OA group or without pre-treated MSC treatment group[19, 20]. The articular cavity environment of patients with OA is different from that of normal people. Inflammatory factors (such as IL-1, TNF), pericellular matrix (such as hyaluronic acid concentration) and an extremely hypoxic environment affect the growth and function of MSCs[7, 32]. In the present study, we observed that KJD + MSCs had better results than the other groups (Figs. 2–5). Especially, we find that the number of CD68+ (macrophage marker) cells significantly decreased more in the affected joint in the KJD + MSCs group than in the other groups (Fig. 5). In this study, we did not find that MSCs directed differentiation to chondrocytes (data not shown). Taken together, we speculated that in this study, MSCs would play a role in the regulation of immunity, and have anti-inflammatory and exocrine effects, which combined with

joint distraction would regulate the micro-environment of the OA joint, facilitating joint intrinsic repair (Figs. 2–5).

There are several mechanisms to explain the treatment of OA by joint distraction. First, the alignment of the joint is corrected to avoid compression of the damaged joint, which is conducive to the repair of the articular surface[7]. Second, joint distraction can generate intermittent hydrostatic pressure, thus stimulating MSCs in the joint cavity to play a therapeutic role[13]. However, the number of MSCs in the articular cavity is relatively small, and it is difficult to stimulate MSCs through joint distraction. Therefore, in current reports of joint distraction treatment of OA, the treatment time is usually longer than two months[25, 33]. In the natural progression of OA, a longer joint distraction cycle will shorten the interval between joint distraction and joint replacement. In order to shorten the joint distraction treatment cycle, in this experiment, we injected MSCs into the articular cavity to increase the number of MSCs. Even though the treatment period of this experiment was only three weeks, which is shorter than that of other experiments, the results showed that the combination KJD + MSCs therapy is better than KJD alone in slowing down the processes of secondary inflammation and subchondral sclerosis and so on (Figs. 2–5). This proves that intra-articular injection of MSCs enhances the therapeutic effect of KJD, and the combined treatment of MSC intra-articular injection and KJD has complementary effects.

Conclusion

In conclusion, in this study, we demonstrated that knee joint distraction combined with mesenchymal stem cell injection can alleviate cartilage degradation, reduce irregular ossification of subchondral bone and secondary inflammation in the rat OA model. This is more effective to delay OA progression than KJD alone.

Abbreviations

ACLT + MMx: Anterior cruciate ligament transection in combination with resection of medial menisci; OA: Osteoarthritis; MSCs: Mesenchymal stem cells; KJD : Knee joint distraction; BMD: Bone mineral density; BV/TV: Bone volume/total volume; Tb.Th.: Trabecular thickness; Tb.Sp.: Trabecular bone space; SMI: Structure model index; MMP13: Matrix metallopeptidase13; Col X: Type X collagen.

Declarations

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was conducted in accordance with the Code of Ethics of the Jinan University. The animal study in this research was approved by the Animal Experimental Ethics Committee of the Jinan University (Ethics Reference No.: 20180824-04).

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CONFLICTS OF INTEREST

No conflicts of interest were stated.

AVAILABILITY OF DATA AND MATERIALS

The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

AUTHOR'S CONTRIBUTIONS

Yuanfeng Chen, Chuanwei Sun and Wenping Liu carried out the study design, animal experiments, data collection, analysis, and manuscript preparation. Yuxin Sun, Sien Lin and Guorong She carried out the animal experiments. Xiaohua Pan carried out manuscript preparation and review. Qiujian Zheng, Kiwai Kevin Ho and Gang Li have contributed to the funding for supporting this research project and supervised all the experiments. All authors read and approved the final manuscript.

CONCENT FOR PUBLICATION

Not applicable.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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Figures

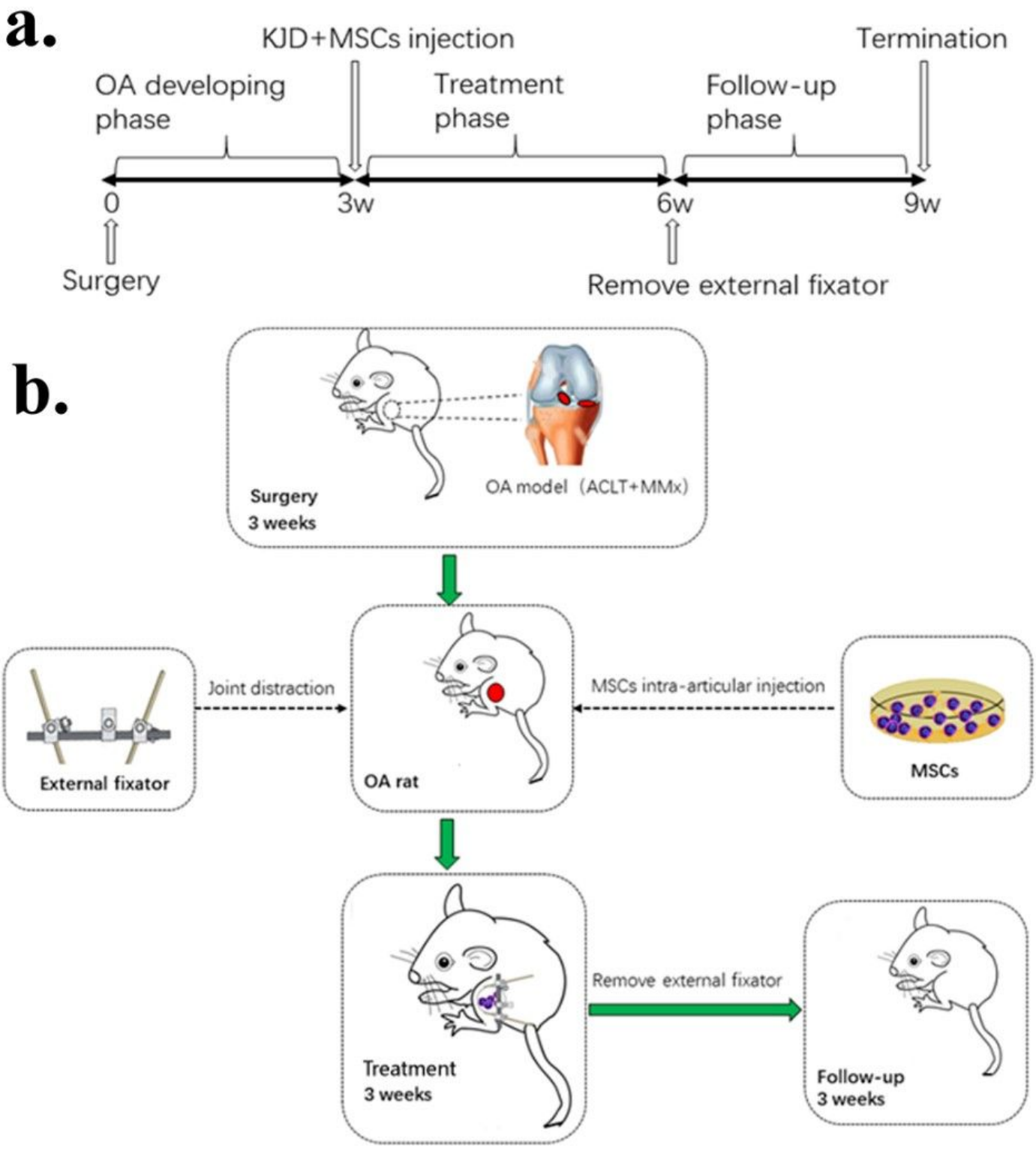

Figure 1

Experimental design. (a) Time schedule (b) Graphic abstract of this experiment.

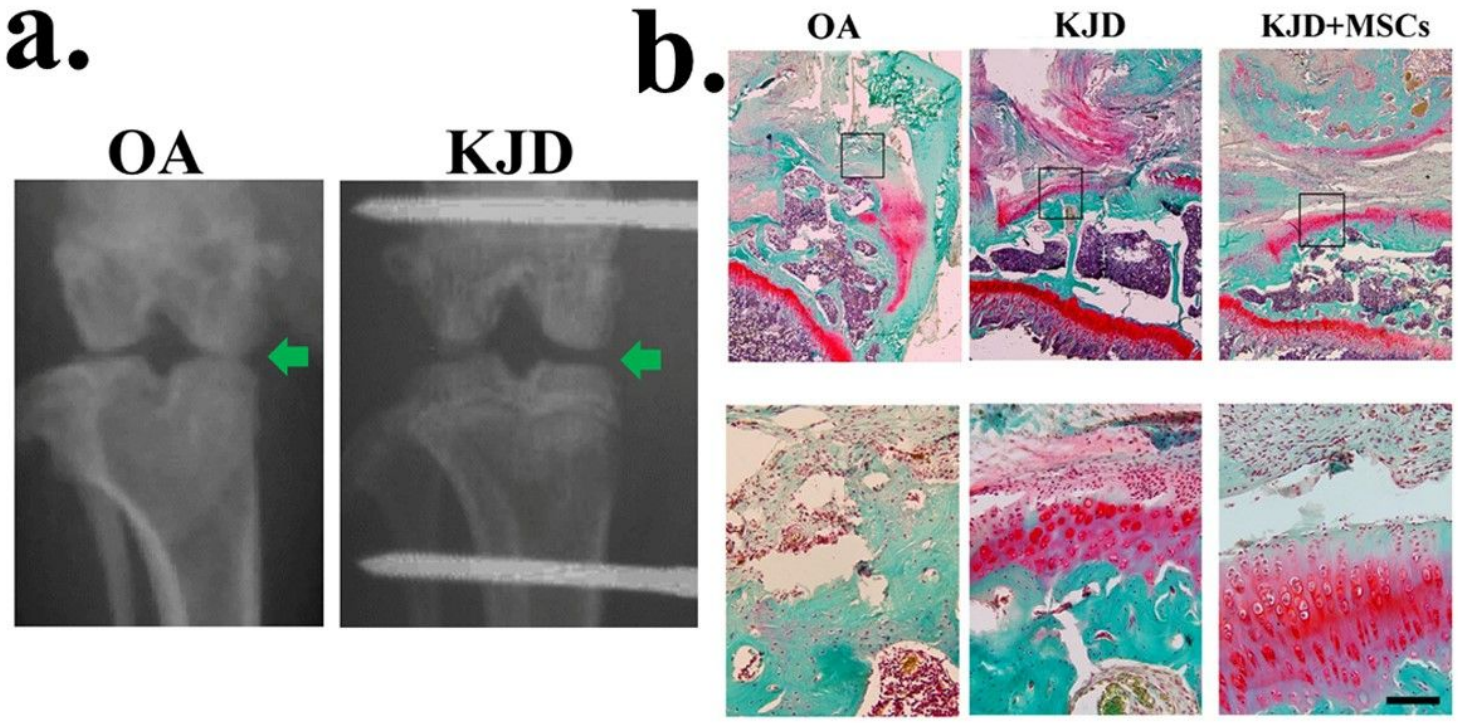

Figure 2

(a) X-ray examination showed that knee joint space was maintained comparably to the contralateral knee in the distraction group. The knee joint space width (green arrow) was significantly increased in the distraction group compared with that in the OA group. (b) Safranin-O/fast green staining showed cartilage lesions in all groups. Cartilage degradation was relieved most effectively in the KJD+MSCs injection group. In the OA and KJD groups, cartilage degradation was severe. Scale: 800 mm (top), 200 mm (bottom).

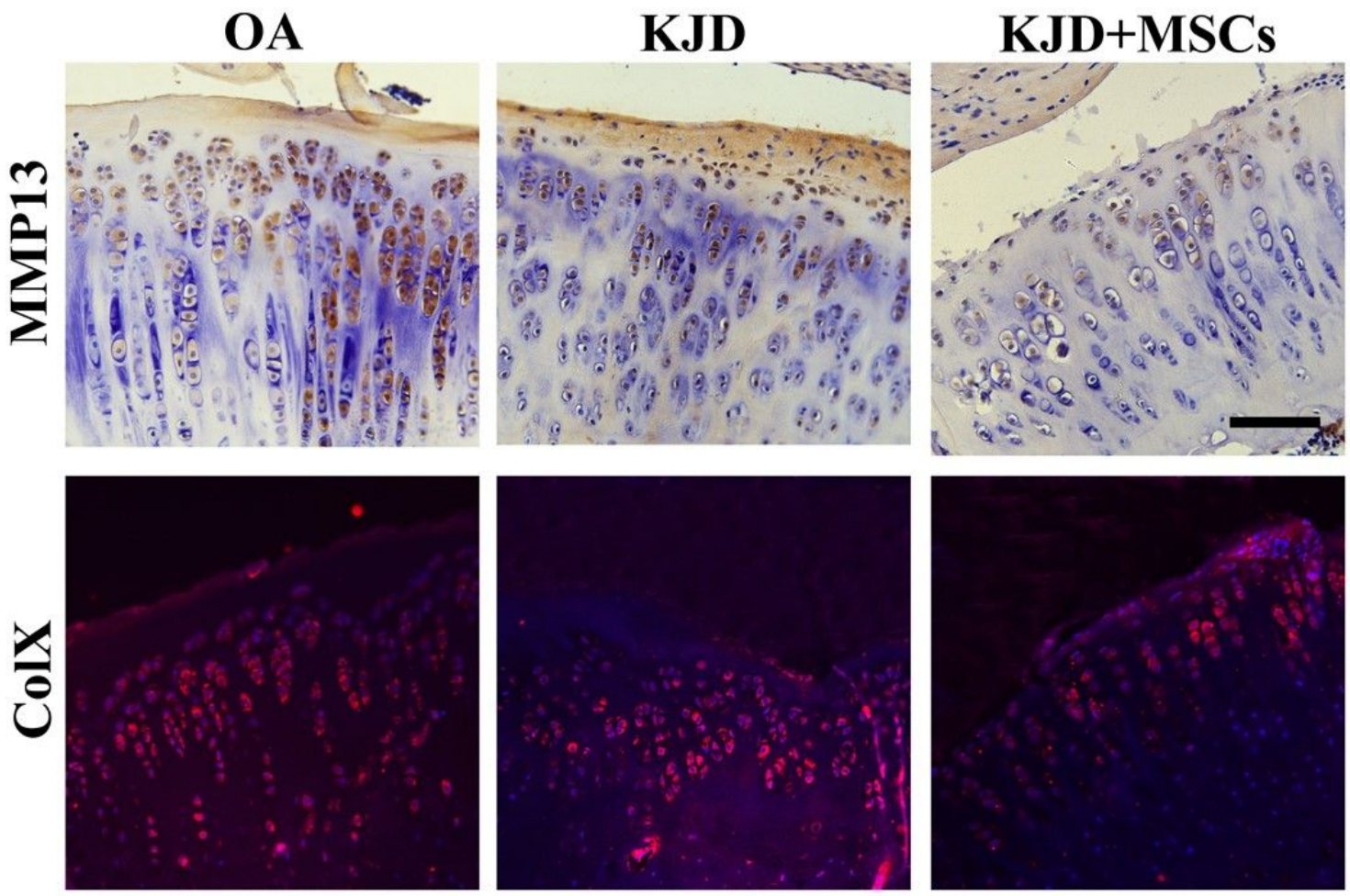

Figure 3

Immunohistochemical staining showed the expression of MMP13 (brown) and Col X (red fluorescence) positive cells in cartilage in the three experimental groups. The number of MMP13 and Col X positive cells in articular cartilage in the KJD+MSCs injection group was significantly less compared with the OA and KJD groups. The nuclei were stained with hematoxylin (black) and DAPI (blue fluorescence). The scale is 100 μm.

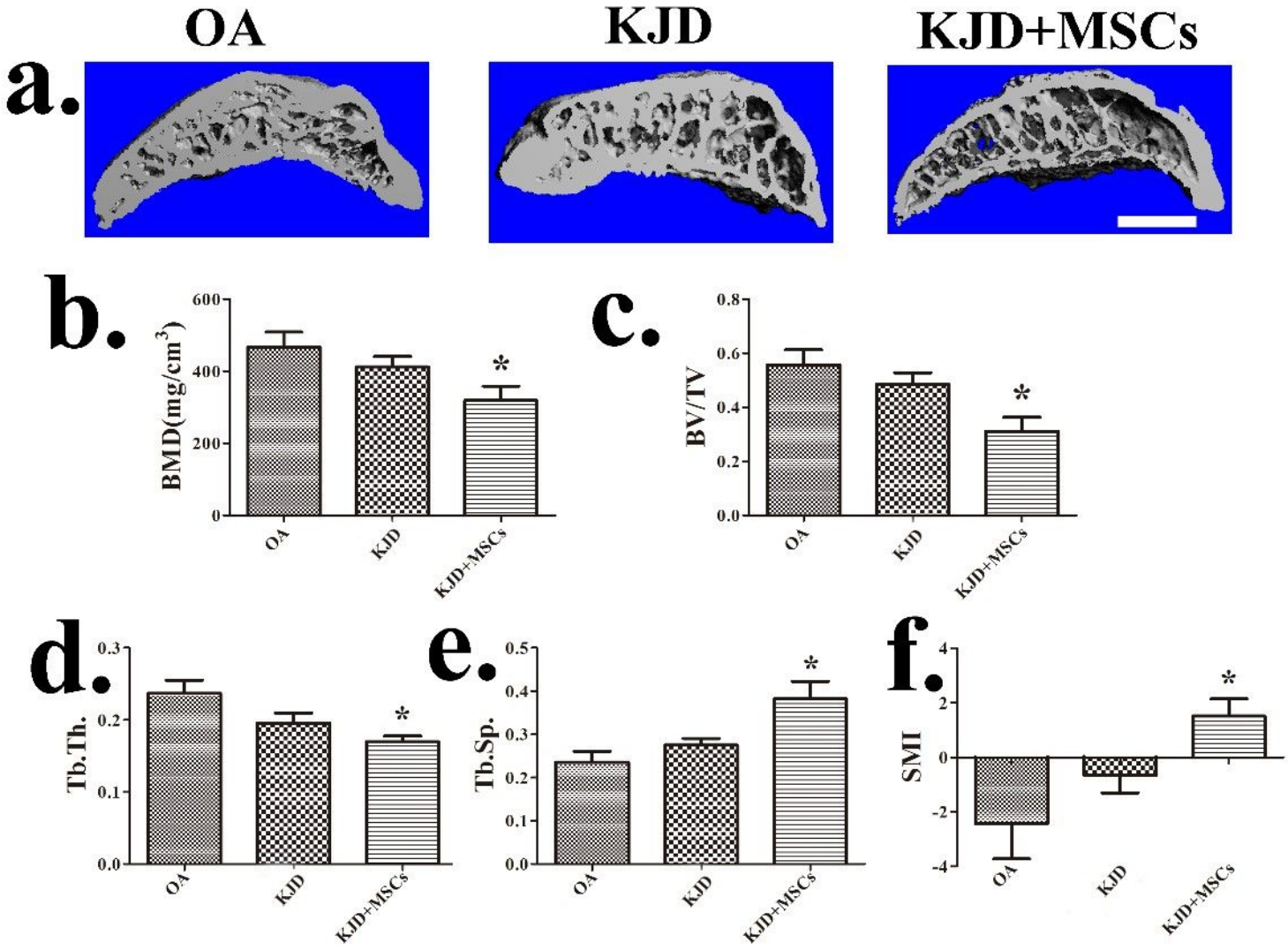

Figure 4

(a) 3D μCT images show the subchondral bone of the tibia in different experimental groups, scale bar 1 mm. Quantitative analysis of subchondral bone microstructure showed: (b) Bone mineral density (BMD), (c) bone volume/total volume (BV/TV), (d) trabecular thickness (Tb.Th.), (e) trabecular bone space (Tb.Sp.) and (f) structure model index (SMI). The results show that BMD, BV/TV and Tb.Th. of subchondral bone in the KJD + MSCs injection group were significantly lower than those in the KJD or OA groups, while Tb.Sp. and SMI increased significantly. * $p < 0.05$: KJD + MSCs versus OA group.

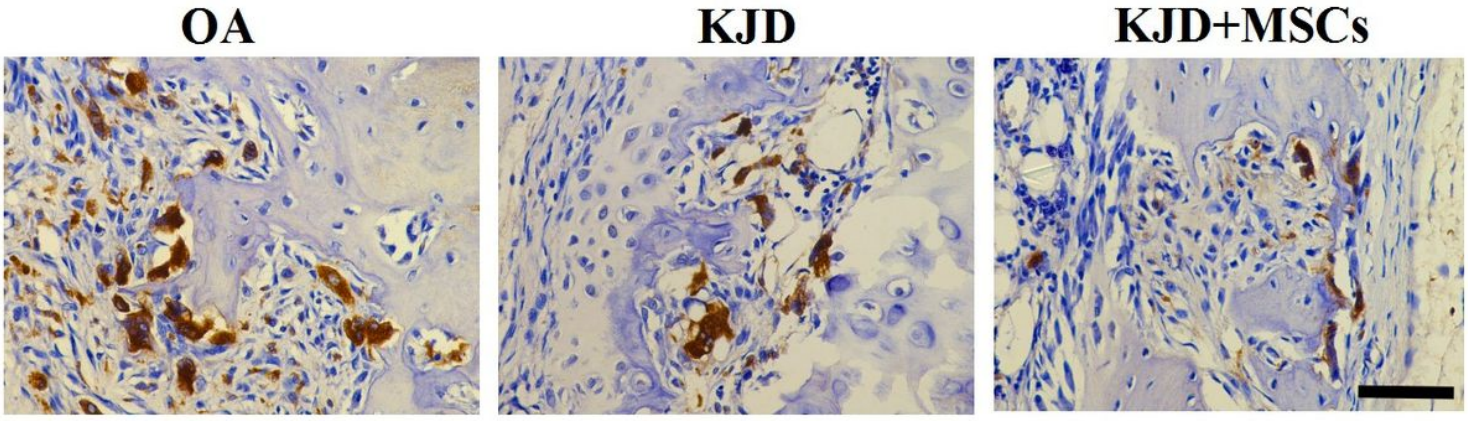

Figure 5

Many cells infiltrated into the knee joint space in all three groups. We used immunohistochemical staining to analyze the number of CD68 positive cells (brown) in the knee joint space in all three groups. The results showed that the fewest CD68 positive cells could be found in the KJD + MSCs injection group. The scale is 50 μm.