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# Joint distraction attenuates osteoarthritis by reducing secondary inflammation, cartilage degeneration and subchondral bone aberrant change

Y. Chen † ‡ [a], Y. Sun † ‡ [a], X. Pan §, K. Ho † ‡ [**], G. Li † ‡ ‖ [*]

† *Department of Orthopaedics & Traumatology, Li Ka Shing Institute of Health Sciences and Lui Che Woo Institute of Innovative Medicine, Faculty of Medicine, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, Hong Kong SAR, PR China*
‡ *The CUHK-ACC Space Medicine Centre on Health Maintenance of Musculoskeletal System, The Chinese University of Hong Kong Shenzhen Research Institute, Shenzhen, PR China*
§ *Department of Orthopaedics and Traumatology, Bao-An District People's Hospital, Shenzhen, PR China*
‖ *Key Laboratory for Regenerative Medicine, Ministry of Education, School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong SAR, PR China*

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SUMMARY

*Objective:* Osteoarthritis (OA) is a progressive joint disorder. To date, there is not effective medical therapy. Joint distraction has given us hope for slowing down the OA progression. In this study, we investigated the benefits of joint distraction in OA rat model and the probable underlying mechanisms.
*Methods:* OA was induced in the right knee joint of rats through anterior cruciate ligament transaction (ACLT) plus medial meniscus resection. The animals were randomized into three groups: two groups were treated with an external fixator for a subsequent 3 weeks, one with and one without joint distraction; and one group without external fixator as OA control. Serum interleukin-1β level was evaluated by ELISA; cartilage quality was assessed by histology examinations (gross appearance, Safranin-O/Fast green stain) and immunohistochemistry examinations (MMP13, Col X); subchondral bone aberrant changes was analyzed by micro-CT and immunohistochemistry (Nestin, Osterix) examinations.
*Results:* Characters of OA were present in the OA group, contrary to in general less severe damage after distraction treatment: firstly, IL-1β level was significantly decreased; secondly, cartilage degeneration was attenuated with lower histologic damage scores and the lower percentage of MMP13 or Col X positive chondrocytes; finally, subchondral bone abnormal change was attenuated, with reduced bone mineral density (BMD) and bone volume/total tissue volume (BV/TV) and the number of Nestin or Osterix positive cells in the subchondral bone.
*Conclusion:* In the present study, we demonstrated that joint distraction reduced the level of secondary inflammation, cartilage degeneration and subchondral bone aberrant change, joint distraction may be a strategy for slowing OA progression.


## Introduction

Osteoarthritis (OA) is a progressive joint disorder characterized by cartilage degeneration, changes in subchondral bone and secondary synovial joint inflammation. Clinical characteristics of OA comprise of pain, stiffness, and functional disabilities[1–3]. Current pharmacological therapy for OA is ineffective at alteration or slowing down the disease progression. Many patients resorted to receiving a total joint arthroplasty at the later stages of their disease and this incident is expected to rise with the advancing age of the

* Address correspondence and reprint requests to: G. Li, Key Laboratory for Regenerative Medicine, Ministry of Education, School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong SAR, PR China.
** Address correspondence and reprint requests to: K. Ho, Department of Orthopaedics & Traumatology, Li Ka Shing Institute of Health Sciences and Lui Che Woo Institute of Innovative Medicine, Faculty of Medicine, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, Hong Kong SAR, PR China.
*E-mail addresses:* kevinho@cuhk.edu.hk (K. Ho), gangli@cuhk.edu.hk (G. Li).
[a] These authors contributed equally to this manuscript.

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general population[4]. Majority of these patients receive their total joint arthroplasty in their 60's and 70's with a high probability that the survivorship of the implants will outlast the life expectancy of the patient. However, there is an increasing trend in which over 40% of all knee replacements and up to 44% of all total knee revisions are performed in patients ≦65 years of age[5]. An alternative strategy is needed in this group of patent who are active with high expectation and high physical demand.

Clinically, joint distraction therapy for OA have given us hope for slowing down the progression of OA and enable intrinsic joint tissue repair by supposedly correcting the proper biochemical and biomechanical joint homeostasis[6]. The principle of joint distraction is a surgical procedure by which two joint surfaces are gradually separated to a certain extent by an external fixator frame for a limited period of time. During this process, further wear and tear of the affected joint is preserved by mechanical unloading[7]. To this date, there have been a few clinical studies for the treatment of OA knee with joint distraction[8–13]. In all these studies, they have demonstrated that the joint space width (JSW) on weight-bearing X-rays had increased after treatment. In a few studies they have also performed arthroscopic evaluation[8,9,12] and/or MRI evaluation[9,10,12] to showed cartilage repair after the joint distraction therapy. Intema *et al.*[10] analyzed biochemical markers for collagen type II turnover and showed an increase of synthesis over release, suggesting that the hyaline nature of the newly formed tissue. However, analyses for changes in subchondral bone or secondary inflammation were not performed in all these studies. Moreover, these studies can only provide an indirect measure of the repair process by way of imaging data or surrogate markers. Therefore, animal studies are needed to evaluate tissue repair directly and in more detail.

In animal studies, several rabbit models have demonstrated the superior repair capacity of joint tissues upon joint distraction[14–17]. But these studies did not measure the changes in subchondral bone or secondary inflammation after treatment. In larger animal model, joint distraction in the canine anterior cruciate ligament transaction (ACLT)-model of OA for 8 weeks resulted in decreased synovial inflammation and normalization of the cartilage matrix turnover as observed directly after treatment[18]. Recently, Wiegant *et al.*[19] reported that they used joint distraction to treat the canine Groove model of OA. In their study the frame was removed at 25 weeks and the subsequent evaluated by histology and biochemistry showed that there was evident of cartilage tissue repair. The researchers have focused mainly on the changes to the articular hyaline cartilage after joint distraction, few reports on the secondary inflammation in OA progression and subchondral bone remodeling.

In the present research we used a rat anterior cruciate ligament transaction plus medial meniscus resection (ACLT + MMx) model of OA to study benefits of joint distraction. We focus on secondary inflammation, cartilage degeneration and changes in subchondral bone, which are known as the characteristic changes of OA. We have also investigated the possible mechanisms of this novel treatment.

## Materials and methods

### *Animal surgery*

All experiments were approved by the Animal Research Ethics Committee at the Chinese University of Hong Kong. A set of 16-week-old male Sprague–Dawley rats, weighting 450–500 g were used in this study. In order to create a post-traumatic OA model, all of these rats were subjected to an anterior cruciate ligament transaction plus medial meniscus resection (ACLT + MMx)[20]. In brief, each rat was anesthetized with a solution of 0.2% (vol/vol) xylazine and 1% (vol/vol) ketamine in PBS, and, after being shaved and disinfected, the right knee joint was exposed through a medial parapatellar approach. The patella was dislocated laterally and the knee placed in full flexion followed by ACL and MCL transection with micro-scissors and resection of the medial meniscus. The surgical incisions were then sutured sequentially.

After 3 weeks of unrestricted activity, these subjects would have display pathological changes consistent with post-traumatic OA[21]. They were then randomly divided into three groups ($n = 5$ each). In the Distraction group, knee joint was distracted for 3 weeks with the use of an external fixator frame. The distraction distance was set at 1 mm. The Fixation group, rats all received an identical external fixator at the knee over the same period of time, but without any distraction. The OA group received no additional treatment to act as the OA control group. In accordance with our animal ethics protocol, all of the animal surgical procedures were performed under general anesthesia and analgesic medication.

### *Joint distraction procedure*

We designed a special external fixation frame [Fig. 1(a)] for the purpose of this experiment. The frame consists of pins, connection junction and the distraction rig. A total of three pins (1.2 mm in diameter) were manually drilled into the medial side of the knee joint; the proximal pin was fixed onto the medial femoral epicondyle. The other two pins were fixed onto the proximal tibia using a special 3-point template. Finally, a custom-made external fixation rig was fastened onto the pins. To ensure the flexion and extension of the knee joint, a cannula (1.3 mm in internal diameter) was placed between the proximal pins and the frame. In the Distraction group, the joint space was widened for 1 mm by the external fixator. We have used the radiography of the contralateral knee as reference [Fig. 1(e)]. Active and passive range of motion (ROM) of the knee joint was observed after the surgery [Fig. 1(b) and (c)]. In the Fixation group, the external fixator was mounted with the knee in extension without distraction. We have used the radiography before and after the application of the external fixator to check the knee joint space to make sure the distraction was applied properly. All of the animals were allowed to move freely during this study.

### *Digital radiographs*

Joint space was monitored using the digital X-ray (MX-20, Faxitron X-Ray Corp., Wheeling, IL, US) under an exposure time of 6000 ms and a voltage of 32 kV. Change in joint space was measured using a calliper on radiograph by two assessors and the average of the measurements was used.

### *Blood collection and serum analysis*

5 ml blood sample was collected by cardiac puncture immediately after the animals were killed. The blood sample was then centrifuged at 1,800 g for 10 min. The resultant sera were then stored at −80°C until analysis. We have used interleuken-1 β as a marker for active inflammation and the levels were measured using the IL-1 β ELISA kit according to the manufacturer's instructions (IL-1 β Elisa kit, lot: EK0393 Boster, USA).

### *Micro-computed tomography (μCT)*

The structural change within the subchondral bone in our model was quantitatively assessed using μCT. At the end of the study, the rats' knees were extracted and dissected without any soft tissue attachment. The specimens were then fixed in 10% formalin before