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

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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

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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

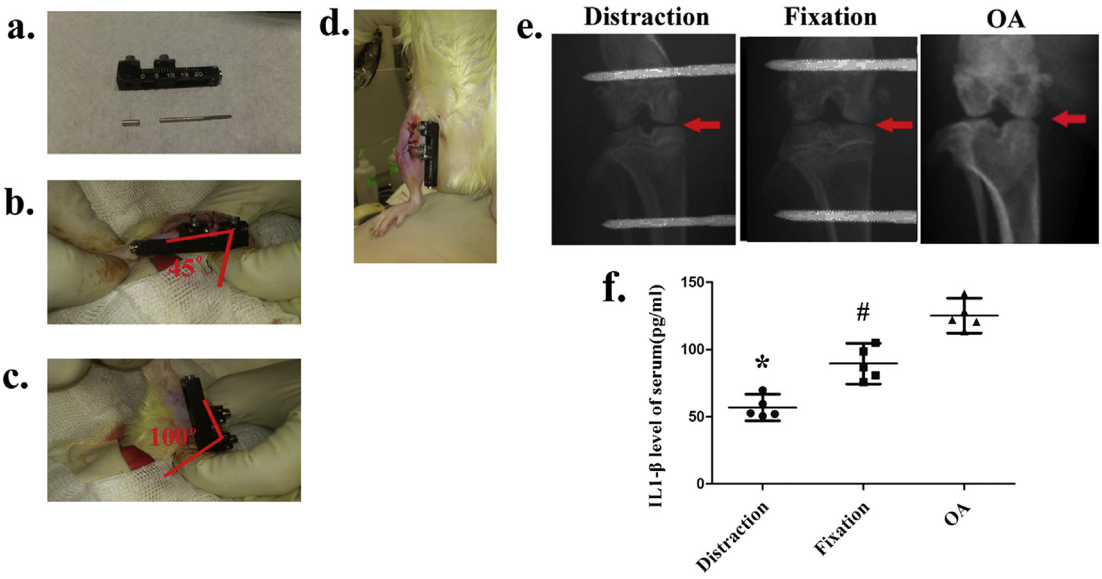

Fig. 1. The joint distraction device helps enlarging the knee joint space. (a) Custom-made external fixator. The external fixator could be lengthened. (b and c) After frame fixation, passive ROM of flexion and extension were recorded. (d) Animal with the external fixator, the frame was fixed in the medial side of the knee joint. (e) X-ray examination showed that knee joint space was maintained similar as the contralateral knee in the fixation group and the distraction group. The OA group was a negative control. The knee JSW (red arrow) was significantly enlarged in the distraction group compared to the fixation group and the OA group. (f) ELISA assay showed the IL1-β level in serum in the joint distraction group significantly reduced compared to the fixation group and OA group (Dot plot of ELISA result showed mean and 95% CI., $n = 5$ per group; $^{*}P < 0.05$: the distraction group compared to the OA group; $\#P < 0.05$: the fixation group compared to the OA group).

analysis with high-resolution μCT (μCT40, Scanco Medical, Bassersdorf, Switzerland) at custom isotropic resolution of 8 μm isometric voxel size with a voltage of 70 kV p and a current of 114 μA. Three dimensional (3-D) reconstructions of mineralized tissues were performed by an application of a global threshold (165 mg hydroxyapatite/cm^3), and a Gaussian filter (sigma = 0.8, support = 2) was used to suppress noise. Sagittal images of the tibiae 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 eight consecutive images from the medial tibial plateau for 3D reconstruction and analysis. After 3D reconstruction, bone mineral density (BMD) and bone volume/total tissue volume (BV/TV) were calculated using built-in software[22].

Histology and immunohistochemistry

The bones including the knee joints were initially fixed in 10% formalin for 48 h and followed by decalcification in 10% EDTA solution for 21 days and embedded into paraffin. Serial 5 μm thick sagittal-oriented sections of the knee joint (medial compartment) were cut at intervals of 0 μm, 100 μm, and 200 μm before mounted onto glass slides[23]. These sections were stained with Safranin-O/fast green. Cartilage degradation was quantified according to Osteoarthritis Research Society International (OARSI) scores[24]. Three independent observers, who were blinded to the experimental groups, scored the sections. Immunostaining was performed using a standard protocol as previously reported. We incubated sections with primary antibodies to rabbit nestin (Sigma., 1:300, N5413), osterix (Abcam, 1:600, ab22552), MMP13 (Abcam, 1:50, ab3208) and collagen X (Abcam, 1:80, ab58632) overnight at 4°C. For immunohistochemical staining, a horse radish peroxidase–streptavidin detection system (Dako, USA) was subsequently used, followed by counterstaining with hematoxylin. Photographs of the selected areas were taken by light microscope (LEICA DMRB, Leica Cambridge Ltd., UK). We counted the number of positively stained cells in the whole tibia cartilage or subchondral bone area per specimen in three sequential sections (0 μm, 100 μm, and 200 μm) per rat in each group, and compared the difference statistically.

Statistical analysis

In accordance with the ARRIVE guidelines[25], we have reported measures of precision, confidence, and *n* to provide an indication of significance. All statistical analyses were performed using the statistical software SPSS15.0. The data were analyzed via one-way ANOVA. The assumptions of the analysis were assessed by the Shapiro–Wilk test of normality and Levene's test for homogeneity of variance. The result of Levene's test was used to determine the *post hoc* testing strategy. If not significant, LSD-t *post hoc* test was employed. If Levene's test was significant, the ANOVA was followed by Dunnett's T3 *post hoc* test for unequal variance. Values of $P < 0.05$ were considered significant. Data were reported as mean and 95% CI (confidence interval). The graphs were generated in GraphPad Prism 6 (GraphPad Software, San Diego, CA, USA).

Results

Joint distraction enlarged the knee joint space

X-ray result of knee joint showed that joint space was significantly enlarged in the joint distraction group compared with that in the Fixation group or the OA group [Fig. 1(e)].

Joint distraction down-regulated IL-1β level in serum of rat OA model

The level of serum IL-1β in the joint distraction group was 65.41 pg/ml, 43.95–71.86 pg/ml, ($n = 5$); which was significantly reduced compared to that of the fixation group [91.67 pg/ml, 70.95–112.39 pg/ml, $n = 5$, $P = 0.055$(the distraction group

compared to the fixation group), one-way ANOVA, Fig. 1(f)] or the OA group [125.96 pg/ml, 107.01–144.92 pg/ml, $P < 0.001$(the distraction group compared to the OA group), one-way ANOVA, Fig. 1(f)].

Joint distraction attenuated cartilage degeneration

The gross macroscopic observation showed that in the OA group, the dissected specimens had rough articular cartilage surfaces and significant osteophyte formation at the intercondylar notch and at the periphery of the joint. In the distraction group, the amount of cartilage damage was much less pronounced. The articular surface of the femoral condyles was relatively smooth and there was no evident of osteophyte formation. In the fixation group, the severity of degenerative changes was at somewhere between the OA and distraction group [Fig. 2(a)].

Similarly, Safranin-O/fast green staining showed that there were significantly more degenerative features in the fixation and OA groups compared to the joint distraction group [Fig. 2(b)]. OARSI scores in both the fixation and OA groups reflected severe cartilage degeneration (12.6, 6.47–18.72 and 14.4, 11.28–17.52, $n = 5$, respectively), whereas cartilage degeneration in the distraction group was significantly less [6.8, 2.07–10.43, $n = 5$, $P = 0.023$(the distraction group compared to the fixation group) and $P = 0.005$(the distraction group compared to the OA group), respectively, one-way ANOVA, Fig. 2(d)].

Moreover, the percentages of MMP13+ chondrocytes were significantly lower in the distraction group (28.16, 14.88–29.76, $n = 5$) than the fixation group (48.4, 41.52–55.28, $n = 5$, $P = 0.005$(the distraction group compared to the fixation group), one-way ANOVA) or the OA group [61.94, 55.31–68.58, $n = 5$, $P < 0.001$(the distraction group compared to the OA group), one-way ANOVA, Fig. 2(c) and (e)]. Likewise, the percentages of type X collagen positive chondrocytes in the distraction group (31.81, 24.57–39.05, $n = 5$) were statistically lower compared to the fixation group (53.17, 43.62–62.72 $n = 5$, $P < 0.001$(the distraction group compared to the fixation group), one-way ANOVA) and OA group [67.62, 62.38–72.86 $n = 5$, $P < 0.001$(the distraction group compared to the OA group), one-way ANOVA, Fig. 2(c) and (f)].

Joint distraction reduced abnormal bone remodeling in subchondral bone areas

The 3D reconstructed images of μCT showed that the microarchitecture of the subchondral bone had significantly changed in the joint distraction group comparing to the OA group. BMD in the distraction group (514.6 mg/cm^3, 505.68–523.51 mg/cm^3, $n = 5$) had significantly decreased than that of the fixation group

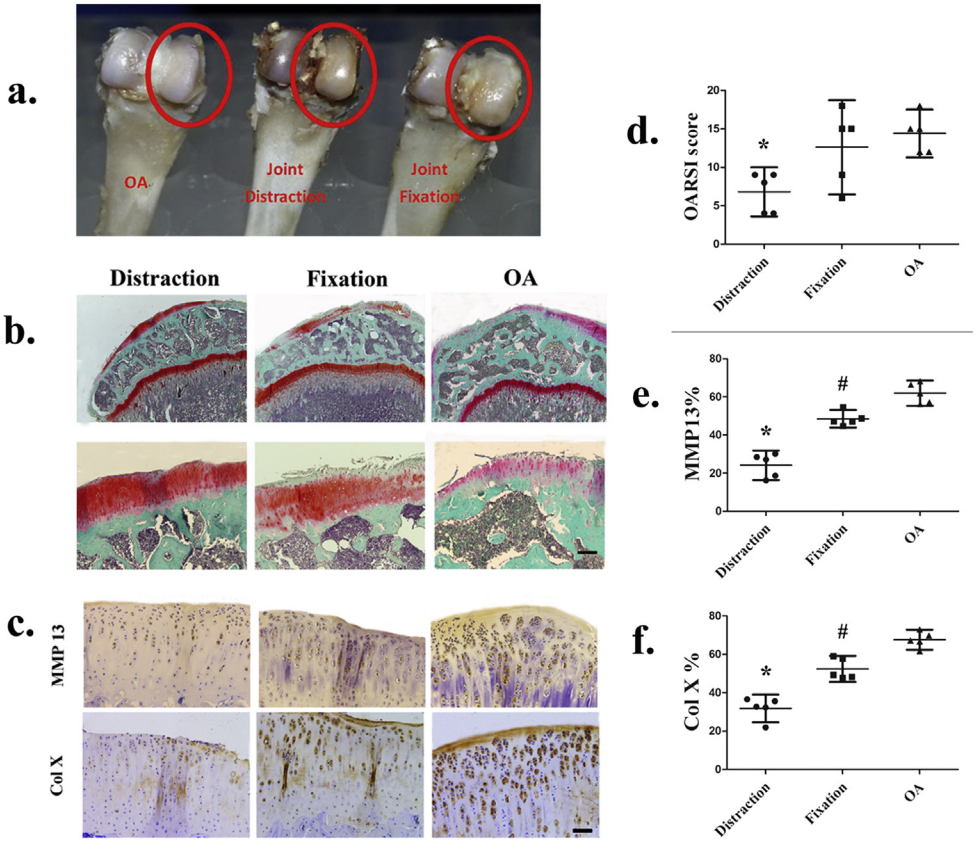

Fig. 2. Joint distraction attenuated articular cartilage degeneration in rat OA model. (a) Gross appearance of femoral condyles in the distraction, fixation and OA groups. In the OA group, the medial condyle of the distal femur showed rough joint surface and exhibited osteophyte formation at the intercondylar space; in the distraction group, joint surface of femoral condyles showed relatively smooth and did not find osteophyte formation; and in the fixation group the joint surface of femoral condyles showed some degree of degeneration and small amount of osteophyte formation; (b) Safranin O and fast green staining of sagittal sections of the subchondral tibia medial compartment in the Distraction group, Fixation group and OA group. Scale bar, 800 μm (in top), 200 μm (in bottom). (d) The OARSI scores showed that cartilage degeneration was most sever in the OA group, followed by fixation and joint distraction groups. (c) Immunohistochemical and (e, f) quantitative analysis of the percentages of MMP13+ and type X collagen (COL X)-positive chondrocytes (brown) in articular cartilage showed that joint distraction group significantly reduced the numbers of MMP13+ and type X collagen (COL X)-positive chondrocytes compared to the other two groups. Scale bars, 100 μm $n = 5$ per group. $^*P < 0.05$: the distraction group compared to the OA group; $\#P < 0.05$: the fixation group compared to the OA group.

(526.06 mg/cm^3, 514.44–537.69 mg/cm^3, $n = 5$, $P = 0.083$(the distraction group compared to the fixation group), one-way ANOVA) and the OA group (538.37 mg/cm^3, 523.87–552.87 mg/cm^3, $n = 5$, $P = 0.002$(the distraction group compared to the OA group), one-way ANOVA). Similarly, BV/TV had distinctly down regulated in the distraction group (0.28, 0.24–0.4, $n = 5$) than that of fixation group (0.35, 0.27–0.37, $n = 5$, $P = 0.16$(the distraction group compared to the fixation group), one-way ANOVA) or the OA group (0.56, 0.54–0.63, $n = 5$, $P < 0.001$(the distraction group compared to the OA group), one-way ANOVA, Fig. 3(a) and (b)).

Notably, the result of immunohistochemistry staining with Nestin, which was expressed primarily in adult bone marrow MSCs[26,27], revealed a significant decreased in the numbers of Nestin positive cells in the subchondral bone marrow in the distraction group (226.8, 191.5–262.1, $n = 5$) compared to that in the fixation group (271, 187.92–377.68, $n = 5$, $P = 0.15$(the distraction group compared to fixation group), one-way ANOVA) or the OA group (433.4, 397.45–540.15, $n = 5$, $P < 0.001$(the distraction group compared to OA group), one-way ANOVA). Once committed to the osteoblast lineage, MSCs express Osterix, a marker of

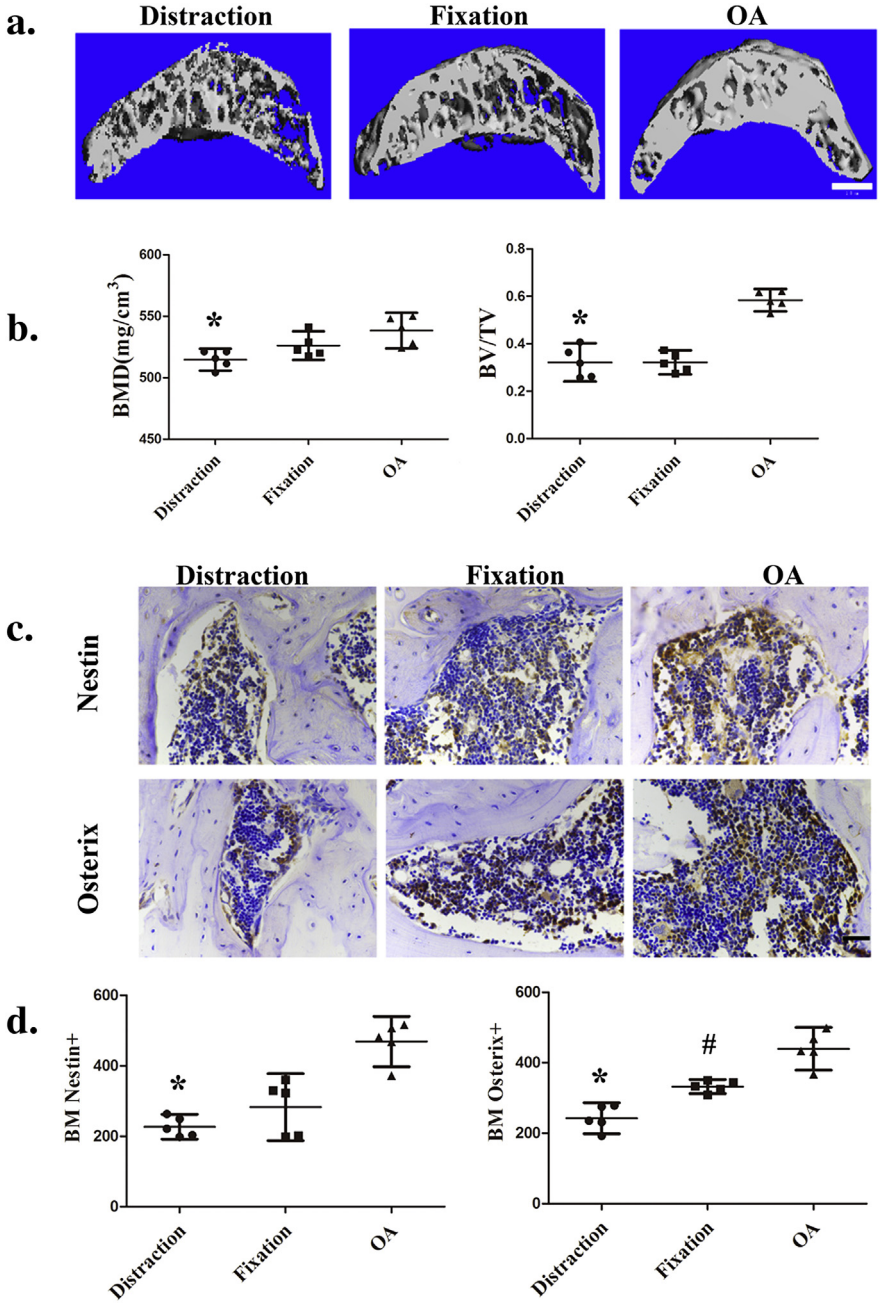

Fig. 3. Joint distraction reduced abnormal bone remodeling in subchondral bone in rat OA model. (a) 3D μCT images of the tibia subchondral bone medial compartment (sagittal view) of rats in the distraction group, fixation group and OA group. Scale bar, 1 mm. (b) Quantitative analysis of structural parameters of subchondral bone by μCT analysis. BMD and BV/TV in subchondral bone determined by μCT. $n = 5$ per group. The results showed that subchondral bone in the OA group had significantly higher BMD and BV/TV in the OA or fixation group comparing to joint distraction group. *$P < 0.05$: the distraction group compared to the OA group. (c–d) Immunohistochemical analysis and quantification of Nestin and Osterix positive cells (brown) in the tibial subchondral region. Joint distraction group significantly reduced Nestin and Osterix positive cells comparing to the other two groups. Scale bars, 50 μm. *$P < 0.05$: the distraction group compared to the OA group; #$P < 0.05$: the fixation group compared to the OA group.

osteoprogenitors. The number of Osterix-positive osteoprogenitors was also significantly down regulated in the subchondral bone marrow in the distraction group (242.2, 198.06–286.34, $n = 5$) compared to that in the fixation group (332.2, 312.11–352.28, $n = 5$, $P = 0.002$(the distraction group compared to fixation group), one-way ANOVA) or the OA group [439.4, 378.55–500.25, $n = 5$, $P < 0.001$(the distraction group compared to OA group), one-way ANOVA, Fig. 3(c) and (d)]. These results suggest that joint distraction would reduce abnormal bone remodeling/formation in the subchondral areas, slowing down the progression of OA.

Discussion

Despite recent medical advancement in diagnosing degenerative joint condition, to date there is no effective therapy that can reverse or halt the progression of OA. The etiological of knee OA is multifactorial with many contributing factors: degenerative, inflammatory, traumatic, mechanical micro-fractures, etc. Aging, joint injury and repetitive excessive joint loading are the well accepted risk factors for OA[28].

Joint injuries, which result in abnormal mechanical stress in joint, dramatically increase the risk of OA, as high as more than 20-fold[29–38]. An increasing evidences showed that joint biological responses to mechanical injuries also play key roles in the onset and progression of OA, including secondary inflammation, cartilage degeneration and changes in subchondral bone remodeling[28,39–43]. Joint distraction through reliving the abnormal mechanical stress could gain long-term clinical benefit for OA patient[8–13]. However, the mechanisms behind this therapy are still poorly understood.

In the present study, we have used an ACLT + MMx OA model to evaluate the effect of joint distraction on OA progression. This model has been well accepted and used previously in many studies. We found that joint distraction could reduce the level of secondary inflammation, cartilage degeneration and subchondral bone aberrant change, unloading the knee joint that allows the cartilage repair or slowing down OA progression. The mechanism is supposedly due to joint distraction reliving the abnormal stress at the joint, allow intrinsic cartilage tissue repair.

Inflammatory cytokine is a main regulator in OA[42,44–52]. Interleukin-1β (IL-1β) is an inflammatory cytokine involves in lubricin catabolism and cartilage degeneration[45–47]. The concentration of stromal cell-derived-factor 1 (SDF-1) has also been related to arthritis development[48]. The role of SDF-1 is to up-regulate matrix metalloproteinase-13 (MMP-13)[49] and accelerate the breakdown of type II collagen and proteoglycans[50]. Wang *et al.* indicated that high fluid shear stress induces pro-inflammatory cytokines PICs and MMPs production via cyclooxygenase-2 (COX-2)-derived prostaglandin PGE2 at the early stage of OA[51]. In a study using cartilage and synovial fluid mononuclear cells collected from OA patient, under intermittent fluid pressure, the expression of catabolic cytokines interleukin 1 and tumor necrosis factor-alpha is proportional to the amount of pressure applied, indicating that mechanical stress plays an important role in inflammatory cell regulation[52]. Wei *et al.* demonstrated that inflammatory factors like SDF-1, IL1β and MMP13 were significantly increased in a rat OA model[42]. In this present study, we found that applying joint distraction significantly reduces the level of IL1β compared to the fixation group and OA control group. One possible mechanism was by relieving the abnormal mechanical stress in the knee joint, which leads to a down-regulation of the inflammatory factors.

Preclinical and clinical studies of OA have focused largely on articular cartilage degenerative changes[28,40,41,53–57]. Recently, researches have shown that the mechanical stress plays an importance role in OA development[28,40,41,56]. *In vitro* studies have demonstrated that acute articular surface impact causes release of reactive oxygen species that lead to chondrocyte cell death[40,56]. Using anti-oxidant N-acetyl-cysteine within 4 h of injury reduced acute chondrocyte cell death by 50%, and prevented longer-term proteoglycan losses[56]. Another research shows that impact injury also leads to release of proteolytic fragments of fibronectin and of type II collagen that induce aggressive chondrolysis with a degree of potency similar to that of pro-inflammatory cytokines[41]. A recent study shows that mechanical stress would stimulate the expression and release of nerve growth factor (NGF) in chondrocytes, suggesting increased mechanical loading in OA joint may mediate OA pain[57]. *In vivo* studies have showed that abnormal mechanical stress in knee joint would lead to cartilage degeneration and overexpression of cartilage degradation markers such as MMP 13 or collagen type X (COL X) in chondrocyte[21,42,43]. In present study, we found that joint distraction would attenuate articular cartilage degeneration in rat OA model; the expression of MMP13 and COL X is dramatically decreased, suggesting that joint distraction would help the chondrocytes in the OA knee to regain their metabolic homeostasis.

The homeostasis and integrity of articular cartilage rely on its biochemical and biomechanical interplay with subchondral bone. The change of subchondral bone plays an importance role in OA progression. Clinically, the characteristics of OA are osteophyte formation, subchondral bone sclerosis, disruption of the tidemark accompanied by angiogenesis at the osteochondral junction and articular cartilage degeneration[58]. Further, bone marrow lesions are closely associated with pain and have been implicated to predict the severity of cartilage damage in OA[59]. Recently, several studies indicate that aberrant mechanical stress plays a role in the change of subchondral bone during OA development[43,60]. Zhen and his colleagues demonstrated that in response to abnormal mechanical loading in an OA mouse model, the transforming growth factor b1 (TGF-b1) was activated in subchondral bone. Higher concentrations of TGF-b1 induced formation of Nestin+ mesenchymal stem cells (MSCs) clusters, leading to formation of marrow osteoid islets accompanied by high levels of angiogenesis[43]. Another study in rat OA model showed that mechanical stress/instability would lead to subchondral stress-induced bone resorption and bone cysts, contributed to OA development[60]. The present findings shows that joint distraction would reduce abnormal bone remodeling in subchondral bone in the rat OA model. In the present study, we also found that in the fixation group the level of abnormal subchondral bone, secondary inflammation or cartilage degeneration were all reduced comparing to that of the OA group; we speculated that the external fixator applied would, to some extent, relieve the abnormal mechanical stress at the knee joint and allow the cartilage recover.

There are limitations in this study. Because the rat has small knee joint and very little synovial fluid, we were unable to collect synovial fluid and test the inflammatory cytokines in the current study. Instead we test the IL-1β level in serum as an indicator for inflammation. Future research should consider using larger animal like rabbit or dog and design collection of synovial fluid and serum at multiple time points to test inflammatory cytokines.

In conclusion, we demonstrated that in rat OA model joint distraction reduces the level of secondary inflammation, cartilage degeneration and subchondral bone aberrant changes, allow the recovery of knee cartilage and slowing down the OA progression.

Conflicts of interest

No conflicts of interest were stated.

Author's contributions

Chen YF and Sun YX carried out all the animal experiments, data collection, analysis and manuscript preparation; Pan XH, Ho KW

and Li G have participated in experimental design, data analysis and manuscript preparation. Pan XH and Li G have contributed to the funding for supporting this research project.

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Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.joca.2015.05.018.

References

1. Bijlsma JW, Berenbaum F, Lafeber FP. Osteoarthritis: an update with relevance for clinical practice. Lancet 2011;377:2115–26.
2. Kinds MB, Welsing PM, Vignon EP, Bijlsma JW, Viergever MA, Marijnissen AC, *et al*. A systematic review of the association between radiographic and clinical osteoarthritis of hip and knee. Osteoarthritis Cartilage 2011;19:768–78.
3. Neogi T, Felson D, Niu J, Nevitt M, Lewis CE, Aliabadi P, *et al*. Association between radiographic features of knee osteoarthritis and pain: results from two cohort studies. BMJ 2009;339:b2844.
4. Hootman JM, Helmick CG. Projections of US prevalence of arthritis and associated activity limitations. Arthritis Rheum 2006;54:226–9.
5. Kurtz SM, Lau E, Ong K, Zhao K, Kelly M, Bozic KJ. Future young patient demand for primary and revision joint replacement: national projections from 2010 to 2030. Clin Orthop Relat Res 2009;467:2606–12.
6. Mastbergen SC, Saris DB, Lafeber FP. Functional articular cartilage repair: here, near, or is the best approach not yet clear? Nat Rev Rheumatol 2013;9:277–90.
7. Lafeber FP, Intema F, Van Roermund PM, Marijnissen AC. Unloading joints to treat osteoarthritis, including joint distraction. Curr Opin Rheumatol 2006;18:519–25.
8. Deie M, Ochi M, Adachi N, Kajiwara R, Kanaya A. A new articulated distraction arthroplasty device for treatment of the osteoarthritic knee joint: a preliminary report. Arthroscopy 2007;23:833–8.
9. Abouheif MM, Nakamura M, Deie M, Adachi N, Nishimori M, Sera S, *et al*. Repair of a large osteochondral defect in the knee joint using autologous and artificial bone graft combined with motion preserving distraction arthroplasty: a case report. Arch Orthop Trauma Surg 2010;130:231–6.
10. Intema F, Van Roermund PM, Marijnissen AC, Cotofana S, Eckstein F, Castelein RM, *et al*. Tissue structure modification in knee osteoarthritis by use of joint distraction: an open 1-year pilot study. Ann Rheum Dis 2011;70:1441–6.
11. Aly TA, Hafez K, Amin O. Arthrodiatasis for management of knee osteoarthritis. Orthopedics 2011;34:e338–343.
12. Wiegant K, van Roermund PM, Intema F, Cotofana S, Eckstein F, Mastbergen SC, *et al*. Sustained clinical and structural benefit after joint distraction in the treatment of severe knee osteoarthritis. Osteoarthritis Cartilage 2013;21:1660–7.
13. Khademi-Kalantari K, Mahmoodi Aghdam S, Akbarzadeh Baghban A, Rezayi M, Rahimi A, Naimee S. Effects of non-surgical joint distraction in the treatment of severe knee osteoarthritis. J Bodyw Mov Ther 2014;18:533–9.
14. Kajiwara R, Ishida O, Kawasaki K, Adachi N, Yasunaga Y, Ochi M. Effective repair of a fresh osteochondral defect in the rabbit knee joint by articulated joint distraction following subchondral drilling. J Orthop Res 2005;23:909–15.
15. Karadam B, Karatosun V, Murat N, Ozkal S, Gunal I. No beneficial effects of joint distraction on early microscopical changes in osteoarthrotic knees. A study in rabbits. Acta Orthop 2005;76:95–8.
16. Yanai T, Ishii T, Chang F, Ochiai N. Repair of large full-thickness articular cartilage defects in the rabbit: the effects of joint distraction and autologous bone-marrow-derived mesenchymal cell transplantation. J Bone Joint Surg Br 2005;87:721–9.
17. Nishino T, Ishii T, Chang F, Yanai T, Watanabe A, Ogawa T, *et al*. Effect of gradual weight-bearing on regenerated articular cartilage after joint distraction and motion in a rabbit model. J Orthop Res 2010;28:600–6.
18. van Valburg AA, van Roermund PM, Marijnissen AC, Wenting MJ, Verbout AJ, Lafeber FP, *et al*. Joint distraction in treatment of osteoarthritis (II): effects on cartilage in a canine model. Osteoarthritis Cartilage 2000;8:1–8.
19. Wiegant K, Intema F, van Roermund PM, Barten-van Rijbroek AD, Doornebal A, Hazewinkel HA, *et al*. Evidence for cartilage repair by joint distraction in a canine model of osteoarthritis. Arthritis Rheumatol 2015;67(2):465–74.
20. Hayami T, Pickarski M, Wesolowski GA, McLane J, Bone A, Destefano J, *et al*. The role of subchondral bone remodeling in osteoarthritis: reduction of cartilage degeneration and prevention of osteophyte formation by alendronate in the rat anterior cruciate ligament transection model. Arthritis Rheum 2004;50:1193–206.
21. Hayami T, Pickarski M, Zhuo Y, Wesolowski GA, RoCdan GA, Duong le T. Characterization of articular cartilage and subchondral bone changes in the rat anterior cruciate ligament transection and meniscectomized models of osteoarthritis. Bone 2006;38:234–43.
22. Peng S, Zhang G, He Y, Wang X, Leung P, Leung K, *et al*. Epimedium-derived flavonoids promote osteoblastogenesis and suppress adipogenesis in bone marrow stromal cells while exerting an anabolic effect on osteoporotic bone. Bone 2009;45:534–44.
23. Wei F, Moore DC, Li Y, Zhang G, Wei X, Lee JK, *et al*. Attenuation of osteoarthritis via blockade of the SDF-1/CXCR4 signaling pathway. Arthritis Res Ther 2012;14:R177.
24. Pritzker KP, Gay S, Jimenez SA, Ostergaard K, Pelletier JP, Revell PA, *et al*. Osteoarthritis cartilage histopathology: grading and staging. Osteoarthritis Cartilage 2006;14:13–29.
25. Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG. Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. Osteoarthritis Cartilage 2012;20:256–60.
26. Mendez-Ferrer S, Michurina TV, Ferraro F, Mazloom AR, Macarthur BD, Lira SA, *et al*. Mesenchymal and haematopoietic stem cells form a unique bone marrow niche. Nature 2010;466:829–34.
27. Wiese C, Rolletschek A, Kania G, Blyszczuk P, Tarasov KV, Tarasova Y, *et al*. Nestin expression–a property of multi-lineage progenitor cells? Cell Mol Life Sci 2004;61:2510–22.
28. Anderson DD, Chubinskaya S, Guilak F, Martin JA, Oegema TR, Olson SA, *et al*. Post-traumatic osteoarthritis: improved

understanding and opportunities for early intervention. J Orthop Res 2011;29:802–9.
29. Davis MA, Ettinger WH, Neuhaus JM, Cho SA, Hauck WW. The association of knee injury and obesity with unilateral and bilateral osteoarthritis of the knee. Am J Epidemiol 1989;130:278–88.
30. Gelber AC, Hochberg MC, Mead LA, Wang NY, Wigley FM, Klag MJ. Joint injury in young adults and risk for subsequent knee and hip osteoarthritis. Ann Intern Med 2000;133:321–8.
31. Bhandari M, Matta J, Ferguson T, Matthys G. Predictors of clinical and radiological outcome in patients with fractures of the acetabulum and concomitant posterior dislocation of the hip. J Bone Joint Surg Br 2006;88:1618–24.
32. Saterbak AM, Marsh JL, Nepola JV, Brandser EA, Turbett T. Clinical failure after posterior wall acetabular fractures: the influence of initial fracture patterns. J Orthop Trauma 2000;14:230–7.
33. Honkonen SE. Degenerative arthritis after tibial plateau fractures. J Orthop Trauma 1995;9:273–7.
34. Volpin G, Dowd GS, Stein H, Bentley G. Degenerative arthritis after intra-articular fractures of the knee. Long-term results. J Bone Joint Surg Br 1990;72:634–8.
35. Weigel DP, Marsh JL. High-energy fractures of the tibial plateau. Knee function after longer follow-up. J Bone Joint Surg Am 2002;84-A:1541–51.
36. Bonar SK, Marsh JL. Unilateral external fixation for severe pilon fractures. Foot Ankle 1993;14:57–64.
37. Kellam JF, Waddell JP. Fractures of the distal tibial metaphysis with intra-articular extension–the distal tibial explosion fracture. J Trauma 1979;19:593–601.
38. Marsh JL, Weigel DP, Dirschl DR. Tibial plafond fractuCres. How do these ankles function over time? J Bone Joint Surg Am 2003;85-A:287–95.
39. Tochigi Y, Buckwalter JA, Martin JA, Hillis SL, Zhang P, Vaseenon T, *et al.* Distribution and progression of chondrocyte damage in a whole-organ model of human ankle intra-articular fracture. J Bone Joint Surg Am 2011;93:533–9.
40. Goodwin W, McCabe D, Sauter E, Reese E, Walter M, Buckwalter JA, *et al.* Rotenone prevents impact-induced chondrocyte death. J Orthop Res 2010;28:1057–63.
41. Ding L, Heying E, Nicholson N, Stroud NJ, Homandberg GA, Buckwalter JA, *et al.* Mechanical impact induces cartilage degradation via mitogen activated protein kinases. Osteoarthritis Cartilage 2010;18:1509–17.
42. Wei L, Fleming BC, Sun X, Teeple E, Wu W, Jay GD, *et al.* Comparison of differential biomarkers of osteoarthritis with and without posttraumatic injury in the Hartley guinea pig model. J Orthop Res 2010;28:900–6.
43. Zhen G, Wen C, Jia X, Li Y, Crane JL, Mears SC, *et al.* Inhibition of TGF-beta signaling in mesenchymal stem cells of subchondral bone attenuates osteoarthritis. Nat Med 2013;19:704–12.
44. Catterall JB, Stabler TV, Flannery CR, Kraus VB. Changes in serum and synovial fluid biomarkers after acute injury (NCT00332254). Arthritis Res Ther 2010;12:R229.
45. Blom AB, van der Kraan PM, van den Berg WB. Cytokine targeting in osteoarthritis. Curr Drug Targets 2007;8:283–92.
46. Jones AR, Flannery CR. Bioregulation of lubricin expression by growth factors and cytokines. Eur Cell Mater 2007;13:40–5. discussion 45.
47. Elsaid KA, Jay GD, Chichester CO. Reduced expression and proteolytic susceptibility of lubricin/superficial zone protein may explain early elevation in the coefficient of friction in the joints of rats with antigen-induced arthritis. Arthritis Rheum 2007;56:108–16.
48. Kanbe K, Takagishi K, Chen Q. Stimulation of matrix metalloprotease 3 release from human chondrocytes by the interaction of stromal cell-derived factor 1 and CXC chemokine receptor 4. Arthritis Rheum 2002;46:130–7.
49. Kanbe K, Takemura T, Takeuchi K, Chen Q, Takagishi K, Inoue K. Synovectomy reduces stromal-cell-derived factor-1 (SDF-1) which is involved in the destruction of cartilage in osteoarthritis and rheumatoid arthritis. J Bone Joint Surg Br 2004;86:296–300.
50. Billinghurst RC, Dahlberg L, Ionescu M, Reiner A, Bourne R, Rorabeck C, *et al.* Enhanced cleavage of type II collagen by collagenases in osteoarthritic articular cartilage. J Clin Invest 1997;99:1534–45.
51. Wang P, Guan PP, Guo C, Zhu F, Konstantopoulos K, Wang ZY. Fluid shear stress-induced osteoarthritis: roles of cyclooxygenase-2 and its metabolic products in inducing the expression of proinflammatory cytokines and matrix metalloproteinases. FASEB J 2013;27:4664–77.
52. van Valburg AA, van Roy HL, Lafeber FP, Bijlsma JW. Beneficial effects of intermittent fluid pressure of low physiological magnitude on cartilage and inflammation in osteoarthritis. An in vitro study. J Rheumatol 1998;25:515–20.
53. Clements DN, Carter SD, Innes JF, Ollier WE, Day PJ. Analysis of normal and osteoarthritic canine cartilage mRNA expression by quantitative polymerase chain reaction. Arthritis Res Ther 2006;8:R158.
54. Welch ID, Cowan MF, Beier F, Underhill TM. The retinoic acid binding protein CRABP2 is increased in murine models of degenerative joint disease. Arthritis Res Ther 2009;11:R14.
55. Zhou J, Chen Q, Lanske B, Fleming BC, Terek R, Wei X, *et al.* Disrupting the Indian hedgehog signaling pathway in vivo attenuates surgically induced osteoarthritis progression in Col2a1-CreERT2; Ihhfl/fl mice. Arthritis Res Ther 2014;16:R11.
56. Martin JA, McCabe D, Walter M, Buckwalter JA, McKinley TO. N-acetylcysteine inhibits post-impact chondrocyte death in osteochondral explants. J Bone Joint Surg Am 2009;91:1890–7.
57. Pecchi E, Priam S, Gosset M, Pigenet A, Sudre L, Laiguillon MC, *et al.* Induction of nerve growth factor expression and release by mechanical and inflammatory stimuli in chondrocytes: possible involvement in osteoarthritis pain. Arthritis Res Ther 2014;16:R16.
58. Suri S, Walsh DA. Osteochondral alterations in osteoarthritis. Bone 2012;51:204–11.
59. Hunter DJ, Zhang Y, Niu J, Goggins J, Amin S, LaValley MP, *et al.* Increase in bone marrow lesions associated with cartilage loss: a longitudinal magnetic resonance imaging study of knee osteoarthritis. Arthritis Rheum 2006;54:1529–35.
60. McErlain DD, Ulici V, Darling M, Gati JS, Pitelka V, Beier F, *et al.* An in vivo investigation of the initiation and progression of subchondral cysts in a rodent model of secondary osteoarthritis. Arthritis Res Ther 2012;14:R26.