



## ■ RESEARCH

# Cyclic compressive loading on 3D tissue of human synovial fibroblasts upregulates prostaglandin E2 via COX-2 production without IL-1 $\beta$ and TNF- $\alpha$

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10.1302/2046-3758.39.2000287  
\$2.00

*Bone Joint Res* 2014;9:280–8.  
Received 13 February 2014;  
Accepted 16 April 2014

## Objective

Excessive mechanical stress on synovial joints causes osteoarthritis (OA) and results in the production of prostaglandin E2 (PGE2), a key molecule in arthritis, by synovial fibroblasts. However, the relationship between arthritis-related molecules and mechanical stress is still unclear. The purpose of this study was to examine the synovial fibroblast response to cyclic mechanical stress using an *in vitro* osteoarthritis model.

## Method

Human synovial fibroblasts were cultured on collagen scaffolds to produce three-dimensional constructs. A cyclic compressive loading of 40 kPa at 0.5 Hz was applied to the constructs, with or without the administration of a cyclooxygenase-2 (COX-2) selective inhibitor or dexamethasone, and then the concentrations of PGE2, interleukin-1 $\beta$  (IL-1 $\beta$ ), tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ), IL-6, IL-8 and COX-2 were measured.

## Results

The concentrations of PGE2, IL-6 and IL-8 in the loaded samples were significantly higher than those of unloaded samples; however, the concentrations of IL-1 $\beta$  and TNF- $\alpha$  were the same as the unloaded samples. After the administration of a COX-2 selective inhibitor, the increased concentration of PGE2 by cyclic compressive loading was impeded, but the concentrations of IL-6 and IL-8 remained high. With dexamethasone, upregulation of PGE2, IL-6 and IL-8 was suppressed.

## Conclusion

These results could be useful in revealing the molecular mechanism of mechanical stress *in vivo* for a better understanding of the pathology and therapy of OA.

**Cite this article:** *Bone Joint Res* 2014;3:280–8.

**Keywords:** Mechanical stress, Osteoarthritis, 3-D culture, Synovial fibroblast, Prostaglandin E2

## Article focus

- Analysis of molecular mechanism of osteoarthritis (OA) development
- Analysis of mechanotransduction in OA development
- Function of synovial fibroblast in OA development

## Key messages

- Cyclic compressive loading on a 3D cultured construct of human fibroblasts upregulated PGE2 via COX-2 production
- Cyclic compressive loading upregulated interleukin-6 (IL-6) and IL-8 proteins
- The expression of these molecules was upregulated without IL-1 $\beta$  and/or tumour necrosis factor (TNF)- $\alpha$  stimulation

## Strengths and limitations

- Strengths - our 3D culture system is close to intra-articular environment
- Our system could be useful in revealing the molecular mechanism of mechanical stress
- Limitation - the intracellular signal transductions of PGE2, IL-6 and IL-8 (mechanotransduction) have not been clarified

## Introduction

Osteoarthritis (OA) is a common disease that causes joint pain, deformity and functional disability, and is increasingly prevalent in hundreds of millions of people worldwide.<sup>1</sup> Congenital disorders, obesity, labour, sports, malalignment and joint instability may

initiate processes leading to loss of cartilage. In addition, repeated excessive mechanical stress on the synovial joint, which is composed of cartilage and synovium, is considered to be a key factor in OA development. However, the molecular relationship between mechanical stress and OA development is still unclear.

OA involves a variable degree of synovitis, and these inflammations cause many symptoms including joint swelling and effusion in clinical situations.<sup>2-6</sup> Synovial fibroblasts and macrophages, as well as chondrocytes, play an important role in OA development through synovitis, and synovial macrophages are considered to produce pro-inflammatory cytokines, such as interleukin-1 $\beta$  (IL-1 $\beta$ ) and tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ).<sup>6-10</sup> These cytokines stimulate synovial fibroblasts and chondrocytes to produce other cytokines, such as IL-6 and IL-8, and several enzymes, such as matrix metalloproteinases (MMPs) and aggrecanases (ADAMTSs). These enzymes sever type II collagen and proteoglycan, the principal components of the extracellular matrix of articular cartilage.<sup>11-15</sup> In addition, prostaglandin E2 (PGE2) plays a significant role in OA by causing pain, inflammation, and cartilage degradation.<sup>16-18</sup> Although PGE2 is known to be produced by synovial fibroblasts or chondrocytes in response to IL-1 $\beta$  and/or TNF- $\alpha$ , which is produced by synovial macrophages,<sup>19-21</sup> the molecular mechanism of PGE2 production triggered by mechanical stress is still unclear.

We have developed a novel three-dimensional (3D) culture system using cyclic mechanical stress on synovial cells or chondrocytes for revealing the molecular mechanism of OA development resulting from mechanical stress. In particular, we have focused on synovial cells, which play an important role in OA development as mentioned above. In our previous study, we have shown that cyclic mechanical stress on 3D cultured constructs of human synovial fibroblasts upregulated mRNA levels of MMP1, MMP2, MMP3, MMP9, MMP13, ADAMTS4, and ADAMTS5 genes in a load-dependent manner<sup>22-23</sup> however, the induction of PGE2 as a result of mechanical stress has not been investigated. In the PGE2 synthesis pathway, cyclooxygenase-2 (COX-2) and microsomal prostaglandin E synthase-1 (mPGES-1) are key enzymes that metabolise arachidonic acid to PGE2.<sup>24,25</sup> Nonsteroidal anti-inflammatory drugs (NSAIDs) and steroids, which downregulate PGE2 synthesis through inhibition of COX-2 activity, have been widely used in the treatment of OA.<sup>19</sup>

The purpose of this study was to examine the expression of PGE2 and the related cytokine expressions of IL-1 $\beta$ , TNF- $\alpha$ , IL-6 and IL-8 by cyclic compressive loading on 3D cultured constructs of human synovial fibroblasts and to clarify the effects of NSAIDs and steroids using our *in vitro* OA model.

## Materials and Methods

### Cell culture of primary human synovial fibroblasts.

Human synovial membranes were obtained aseptically from eight patients aged from 17 to 34 years (three male,

five female) who underwent arthroscopic knee surgery in accordance with a protocol approved by the Osaka University Institutional Ethical Committee. We followed the Helsinki Declaration and obtained written informed consent from all the patients involved in this study. The cell isolation protocol was essentially the same as the protocol used previously for the isolation of human synovial fibroblasts.<sup>22,26</sup> In brief, synovial membrane specimens were rinsed with phosphate-buffered saline (PBS), minced meticulously and digested with 0.4% collagenase XI (Sigma-Aldrich, St. Louis, Missouri) for two hours at 37°C. After neutralisation of the collagenase with a growth medium containing high-glucose Dulbecco's Modified Eagle's Medium (HG-DMEM, Wako, Osaka, Japan) supplemented with 10% fetal bovine serum (FBS; HyClone, Logan, Utah) and 1% penicillin/streptomycin (Gibco BRL, Life Technologies Inc., Carlsbad, California), the cells were collected by centrifugation, washed with PBS, resuspended in a growth medium, and plated in culture dishes. For expansion, cells were cultured in the growth medium at 37°C in a humidified atmosphere of 5% CO<sub>2</sub>. The medium was replaced once a week. After ten to 14 days of primary culture, when the cells reached near confluence, they were washed twice with PBS, harvested by treatment with trypsin-EDTA (0.25% trypsin and 1 mM EDTA; Gibco BRL, Life Technologies Inc.), and replated at 1:3 dilution for the first subculture. Cell passages were continued in the same manner with 1:3 dilution when cultures reached near confluence. Cells at passages 3 to 7 were used in the present study.

**Cell seeding on collagen scaffold and production of the 3D engineered construct.** The primary cultured cells were harvested and seeded on collagen scaffolds to produce 3D constructs as previously described.<sup>22,23</sup> In brief, the cultured cells ( $5 \times 10^5$ /scaffold) were suspended in a growth medium and then mixed with an equal volume of 1% Atelocollagen gel (Koken, Tokyo, Japan) on ice to produce a cell suspension in 0.5% collagen solution. The cell suspension was incorporated into collagen scaffolds (Atelocollagen Sponge Mighty, Koken, Tokyo, Japan; 5 mm diameter, 3 mm thick) by centrifugation at  $500 \times g$  for five minutes. The collagen scaffold which we used has an interconnected pore size of 30 nm to 200 nm. The scaffolds were fabricated via the process of freeze-drying of 10% collagen gel and cross-linking to reinforce the mechanical property. This is similar to those of articular cartilage. The cell-scaffold constructs were then incubated at 37°C for gelation to produce 3D cell-scaffold constructs (Fig. 1a). The cells in the 3D construct were evenly embedded in the collagen scaffold, with no cell leakage and collagen breakage after cell seeding, as we have previously shown with histological evaluation.<sup>22</sup> The constructs were maintained in a growth medium of HG-DMEM, with 10% FBS in free-swelling conditions at 37°C and in 5% CO<sub>2</sub> for three days prior to the application of cyclic load stimulation.

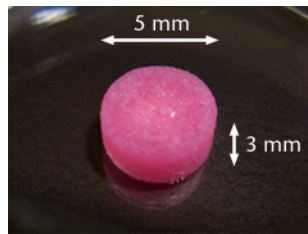


Fig. 1a



Fig. 1b

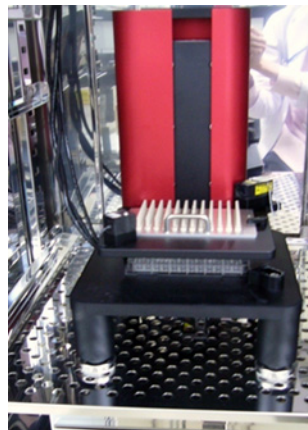


Fig. 1c

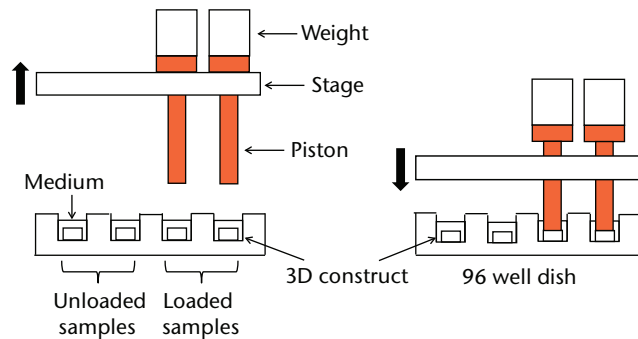


Fig. 1d

Figure 1a – 3D cell-scaffold constructs made using collagen scaffolds (AtelloCell, MIGHTY); b) monitor and controller; c) Cyclic load stimulator (CLS-5J-Z, Technoview, Osaka, Japan) in the incubator; d) Schematic representation of the cyclic load stimulator, cyclic-loaded samples, and unloaded samples; e) Experimental protocol for cyclic compressive loading on 3D constructs.

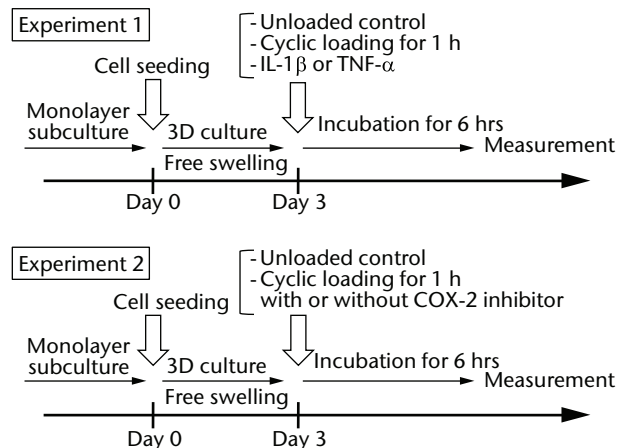


Fig. 1e

**Cyclic compressive loading on 3D constructs.** Cyclic unconfined compressive loading was applied to the 3D constructs using a custom-designed apparatus, a cyclic load bioreactor (CLS-5J-Z, Technoview, Osaka, Japan), as previously described (Figs 1b to 1d).<sup>23</sup> In brief, the loading experiments were performed with metal platens and plastic culture dishes in HG-DMEM and 10% FBS in a humidified incubator maintained at a temperature of 37°C in 5% CO<sub>2</sub>. In all of these experiments, a cyclic compressive load of 40 kPa was applied to the constructs for one hour at the rate of 0.5 Hz, in accordance with the protocol used previously, in order to detect the expression of PGE<sub>2</sub>, IL-1β, IL-6, IL-8 and TNF-α more easily.<sup>22,23</sup> As mentioned above, a cyclic compressive load of 40 kPa was chosen, which yielded a 10% compression strain (approximately),

because it maximally induced the mRNA expression of MMP1, MMP3, MMP9, MMP13 genes compared with the lower compressive loading of 0 kPa or 20 kPa in our previous study.<sup>22</sup> In addition, we measured the expression of PGE<sub>2</sub> and the related cytokine expressions six hours after cyclic loading according to our previous study, in which the expression of MMPs maximally upregulated at this time.<sup>22</sup>

**Experimental design.** The experimental design is illustrated in Fig. 1e. On day 0, the primary cultured human synovial fibroblasts were harvested, seeded on collagen scaffolds, and maintained in growth media for three days in free-swelling conditions. For the first experiment, on day three, cyclic compressive loading was applied to the 3D constructs for one hour. 3D constructs without

loading were considered to be the control. After six hours, culture supernatant was collected, and the concentrations of PGE<sub>2</sub>, IL-1 $\beta$ , TNF- $\alpha$ , IL-6 and IL-8 were measured with the homogeneous time-resolved fluorescence (HTRF) method (described below in detail). In addition, the mRNA expression of COX-2 and mPGES-1 genes were quantitatively measured using a real-time polymerase chain reaction (PCR). In contrast to the cyclic loading, 10 ng/ml of IL-1 $\beta$  (R&D Systems, Minneapolis, Minnesota) or 100 ng/ml of TNF- $\alpha$  (R&D Systems) was administered to the unloaded 3D constructs on day three. A total of six hours after the administration of these cytokines, the concentration of PGE<sub>2</sub> in culture supernatant was measured using HTRF. For the second experiment, cyclic compressive loading was applied to the 3D constructs with or without two types of COX-2 inhibitors: COX-2 selective inhibitor (celecoxib, provided by Pfizer Japan Inc., Tokyo, Japan) or dexamethasone (Sigma-Aldrich). These drugs were administered just before cyclic compressive loading was applied. Six hours after cyclic loading, the concentrations of PGE<sub>2</sub>, IL-6 and IL-8 in culture supernatant were measured using HTRF. In addition, the mRNA expression of the COX-2 gene was quantitatively estimated by real-time PCR.

**Quantitative protein analysis of culture supernatant using HTRF.** For each culture supernatant sample, an enzyme immunoassay was performed to measure the concentrations of PGE<sub>2</sub>, IL-1 $\beta$ , TNF- $\alpha$ , IL-6 and IL-8 using HTRF human PGE<sub>2</sub>, IL-1 $\beta$ , TNF- $\alpha$ , IL-6 and IL-8 assay kits (CIS Bio International, Saclay, France).

**Quantitative mRNA expression analysis of COX-2 and mPGES-1 genes.** Total RNAs from the 3D constructs were extracted using a RNeasy mini kit (Qiagen, Valencia, California). Complementary DNAs (cDNAs) were obtained by the use of a reverse transcription (RT) of 200  $\mu$ g of total RNA through the use of a reverse transcription system (Promega, San Luis Obispo, California) with random primers. For the quantification of gene expression, PCR amplification was performed with SYBR Premix ExTaq (Takara Bio, Shiga, Japan) on a LightCycler 1.5 real-time PCR system (Roche, Indianapolis, Indiana). RNA expression levels were normalised to that of GAPDH. The primers used were as follows: human GAPDH (forward): TCT CTG CTC CTC CTG TTC GAC, (reverse): GTT GAC TCC GAC CTT CAC CTT C, human COX-2 (forward): AGG GTT GCT GGT GGT AGG AA, (reverse): GGT CAA TGG AAG CCT GTG ATA CT, human mPGES-1 (forward): CCT GGG CTT CGT CTA CTC CTT, (reverse): AGT GCA TCC AGG CGA CAA A.

**Statistical analysis.** Every experiment was performed more than three times using independent donors. Statistical analysis was performed with analysis of variance (ANOVA) followed by *post hoc* testing (> 2 groups). The comparison of other parameters was analysed with a Mann-Whitney U test (two groups). The results are presented as mean and SD. The data were analysed with JMP 9 (SAS Institute, Cary, North Carolina) and significance was set at  $p < 0.05$ .

## Results

**The expressions of PGE<sub>2</sub> and related molecules by cyclic compressive loading.** The concentrations of PGE<sub>2</sub>, IL-6 and IL-8 in a culture supernatant of loaded samples were significantly higher compared with that of unloaded samples (PGE<sub>2</sub>, 0.33 ng/ml (SD 0.055) vs 2.07 ng/ml (SD 0.65),  $p < 0.01$  (Fig. 2a); IL-6, 0.71 ng/ml (SD 0.42) vs 6.89 (SD 0.25),  $p < 0.01$  (Fig. 2b); and IL-8, 0.77 ng/ml (SD 0.39) vs 8.76 ng/ml (SD 0.69),  $p < 0.01$  (Fig. 2c)). However, the concentrations of IL-1 $\beta$  and TNF- $\alpha$  were unchanged between loaded and unloaded samples (IL-1 $\beta$ , 4.8 pg/ml (SD 8.2) vs 7.4 pg/ml (SD 8.4),  $p = 0.74$  (Fig. 2d) and TNF- $\alpha$ , 9.6 pg/ml (SD 8.8) vs 7.6 pg/ml (SD 8.3),  $p = 0.75$  (Fig. 2e)). The administration of IL-1 $\beta$  or TNF- $\alpha$  also significantly induced PGE<sub>2</sub> production compared with the non-administered control (IL-1 $\beta$ , 0.33 ng/ml (SD 0.055) vs 2.25 ng/ml (SD 0.65),  $p < 0.01$  and TNF- $\alpha$ , 0.33 ng/ml (SD 0.055) vs 1.84 ng/ml (SD 0.63),  $p < 0.01$  (Fig. 2a)). The mRNA levels of COX-2 and mPGES-1 genes of loaded samples were significantly higher compared with that of unloaded samples (COX-2, 1 vs 6.97 (SD 3.66),  $p < 0.01$  (Fig. 2f); mPGES-1, 1 vs 5.03 (SD 2.94),  $p < 0.01$  (Fig. 2g)).

**The effects of a COX-2 selective inhibitor on mechanically induced PGE<sub>2</sub>, IL-6 and IL-8 proteins and COX-2 gene expressions.** The increased concentration of PGE<sub>2</sub> by cyclic compressive loading was impeded in a dose-dependent manner after administration of a COX-2 selective inhibitor (Fig. 3a). More than 100 nM of a COX-2 selective inhibitor significantly abolished the upregulation of PGE<sub>2</sub> by cyclic compressive loading ( $p < 0.01$ ). However, the increased concentration of IL-6 and IL-8 by cyclic compressive loading remained high, and the inhibitory effects of the COX-2 selective inhibitor were not observed (Figs 3b and 3c). The upregulation of COX-2 mRNA levels by cyclic compressive loading was not suppressed by a COX-2 selective inhibitor (Fig. 3d).

**The effects of dexamethasone on mechanically induced PGE<sub>2</sub>, IL-6 and IL-8 proteins and COX-2 gene expressions.** The increased concentration of PGE<sub>2</sub> by cyclic compressive loading was suppressed in a dose-dependent manner after administration of dexamethasone (Fig. 4a). More than 100 nM of dexamethasone significantly abolished the upregulation of PGE<sub>2</sub> by cyclic compressive loading ( $p < 0.01$ ). Similarly, the increased concentration of IL-6 and IL-8 was also suppressed in a dose-dependent manner (Figs 4b and 4c). More than 100 nM of dexamethasone significantly abolished the upregulation of IL-6 or IL-8 by cyclic compressive loading ( $p < 0.01$ ). The upregulation of COX-2 mRNA levels by cyclic compressive loading was suppressed in a dose-dependent manner after the administration of dexamethasone (Fig. 4d). More than 10 nM of dexamethasone significantly abolished the upregulation of COX-2 mRNA levels by cyclic compressive loading ( $p < 0.01$ ).

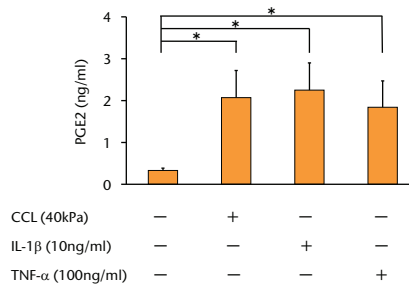


Fig. 2a

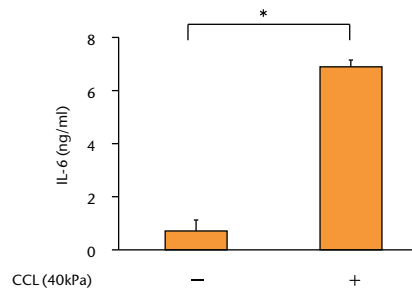


Fig. 2b

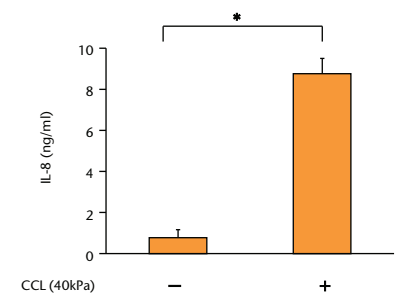


Fig. 2c

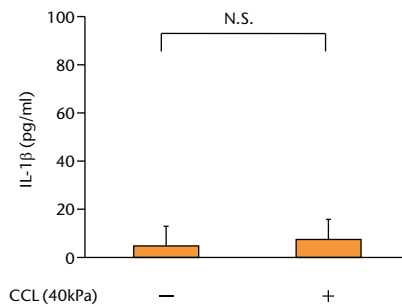


Fig. 2d

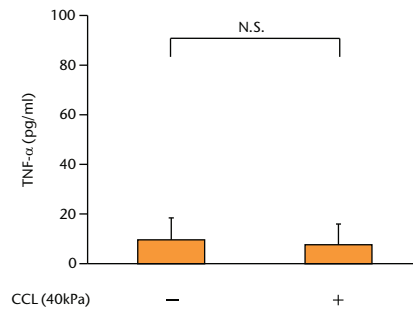


Fig. 2e

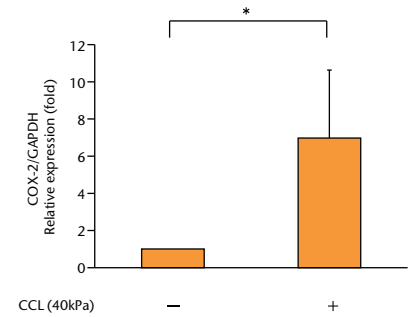


Fig. 2f

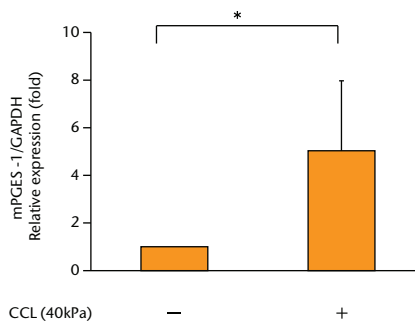


Fig. 2g

Graphs showing the expressions of PGE2 and related molecules by cyclic compressive loading. a) PGE2 was significantly upregulated by cyclic compressive loading, interleukin- (IL-)1β, or tumour necrosis factor- (TNF)-α (n = 7). b) IL-6 (n = 6) and c) IL-8 (n = 6) were significantly upregulated by cyclic compressive loading. d) IL-1β (n = 6) and e) TNF-α (n = 6) were not upregulated by cyclic compressive loading. f) COX-2 (n = 6) and g) mPGES-1 (n = 5) mRNA levels were significantly upregulated by cyclic compressive loading (CCL;cyclic compressive loading)\*p < 0.01.

## Discussion

Mechanical stress is believed to be important for every cell in our body, particularly intra-articular tissues such as bone, cartilage, meniscus, and synovium, for the maintenance and regeneration of tissues and organs. Many studies have demonstrated that mechanical stress to chondrocytes, cartilage explants, or mesenchymal stem cells promoted bone and cartilage development.<sup>27-30</sup> However, mechanical stress causes joint diseases, and excessive mechanical stress may lead to the development of OA. PGE2 is well known as a pathogenic molecule related to OA development, in addition to MMPs, ADAMTS, and inflammatory cytokines.<sup>31</sup> To investigate the molecular mechanisms of PGE2 and related inflammatory cytokines by mechanical stress, we used the 3D culture system using cyclic compressive loading, which

can mimic the intra-articular environment through the adjustment of magnitudes, durations, and frequencies of loads. The loading condition of this study represents that cyclic loading for one hour at a rate of 0.5 Hz is nearly equal to the walking pace. We have chosen 40 kPa, which yielded approximately 10% compression strain, because it maximally induced mRNA expression of MMP1, MMP3, MMP9 and MMP13 genes compared with the lower compressive loading of 0 kPa or 20 kPa in our previous study.<sup>22</sup> In addition, there have been no obvious data of biomechanics in synovium as far as we know, while > 10% compression strain to cartilage was shown to inhibit proteoglycan and protein synthesis in a dose-dependent manner in bovine calf cartilage.<sup>32-34</sup> Therefore, this loading condition may be considered excessive loading over the physiological conditions. Also, the

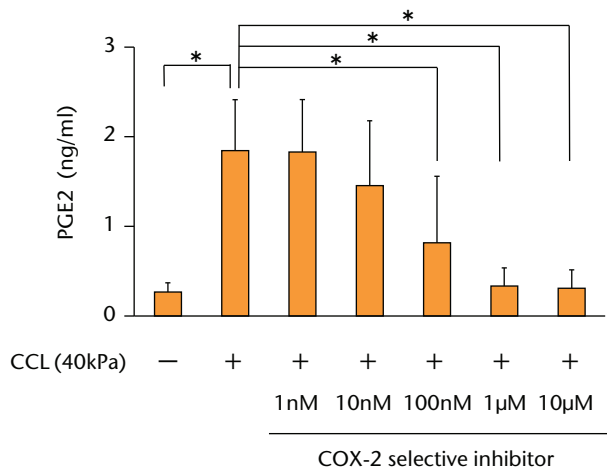


Fig. 3a

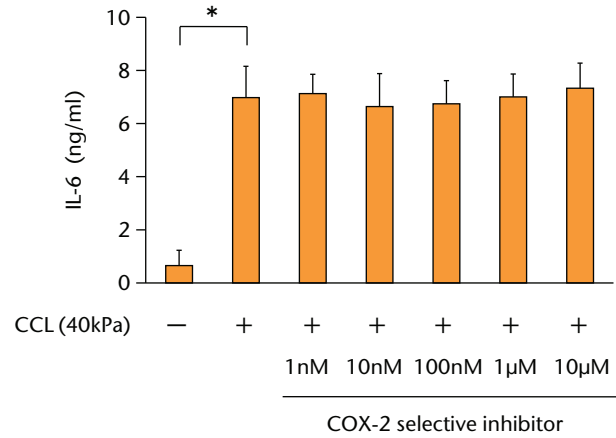


Fig. 3b

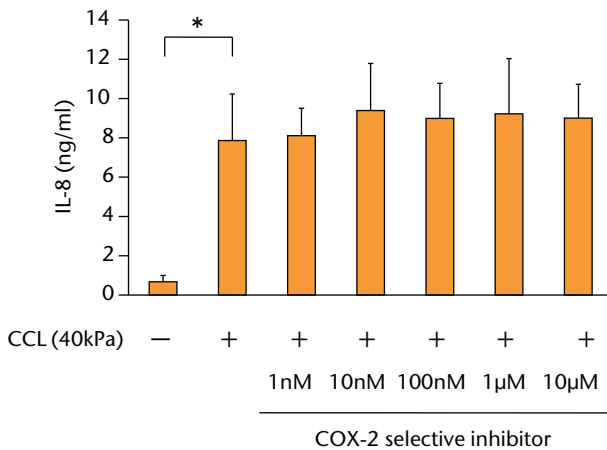


Fig. 3c

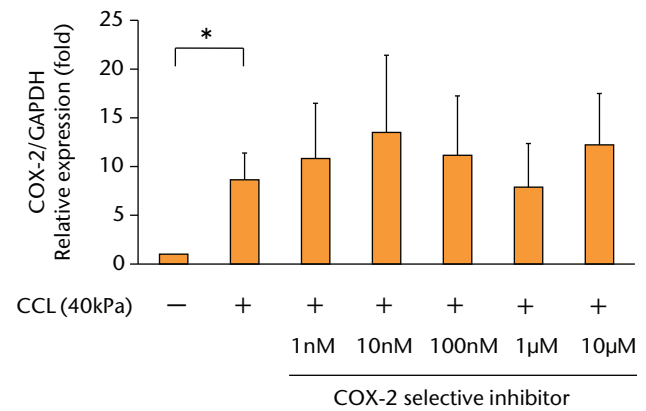


Fig. 3d

a) The increased concentration of PGE2 by cyclic compressive loading was impeded in a dose-dependent manner ( $n = 8$ ). b) The increased concentrations of IL-6 ( $n = 7$ ) and c) IL-8 ( $n = 5$ ) by cyclic compressive loading remained high. d) The upregulation of COX-2 mRNA levels by cyclic compressive loading was not suppressed by a COX-2 selective inhibitor ( $n = 5$ ). \* $p < 0.01$

loading was applied with uni-axial unconfined compression, and this condition could also mimic the intra-articular environment, in which both compressive and tensile stresses are applied to the synovium.<sup>23,35,36</sup> Moreover, a 3D culture system is better to evaluate a biological reaction, because 3D culture is close to the physical environment and there are sometimes differences detected between 2D and 3D cultures.<sup>37-41</sup>

In this study, we directly demonstrated that cyclic compressive loading on a 3D-cultured construct of human synovial fibroblasts upregulated PGE2, IL-6 and IL-8 proteins. We also showed that the gene expression of COX-2 and mPGES-1, which are the key enzymes that metabolise arachidonic acid to PGE2, was upregulated by cyclic compressive loading (Fig. 5). In addition, the upregulation of PGE2 by cyclic compressive loading was

suppressed by the administrations of a COX-2 selective inhibitor or dexamethasone in a dose-dependent manner. As a pharmacological effect, COX-2 selective inhibitors inhibit the activity of COX-2, whereas dexamethasone inhibits the synthesis of COX-2.<sup>42-45</sup> In this study, a COX-2 selective inhibitor suppressed PGE2 production in a dose-dependent manner without changing the COX-2 mRNA level, whereas dexamethasone suppressed PGE2 production by suppressing the COX-2 gene expression. These results reflect well with the pharmacology of PGE2 inhibition by NSAIDs and steroids in OA. Interestingly, a COX-2 selective inhibitor did not suppress IL-6 and IL-8 production, whereas dexamethasone suppressed these cytokines in a dose-dependent manner. The different effects of these chemicals on IL-6 and IL-8 may account for the distinct functions in clinical usage.

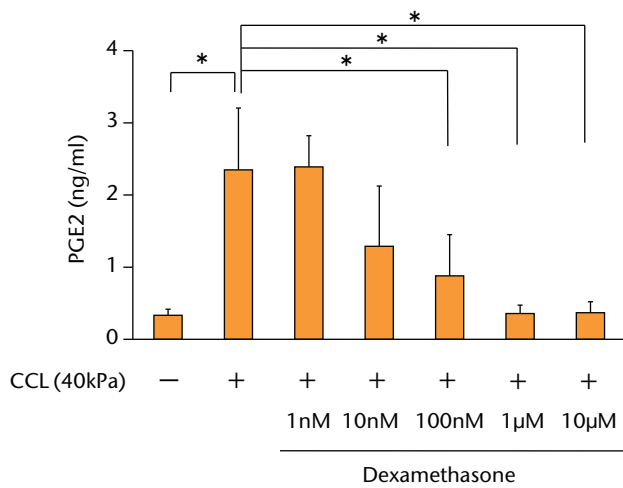


Fig. 4a

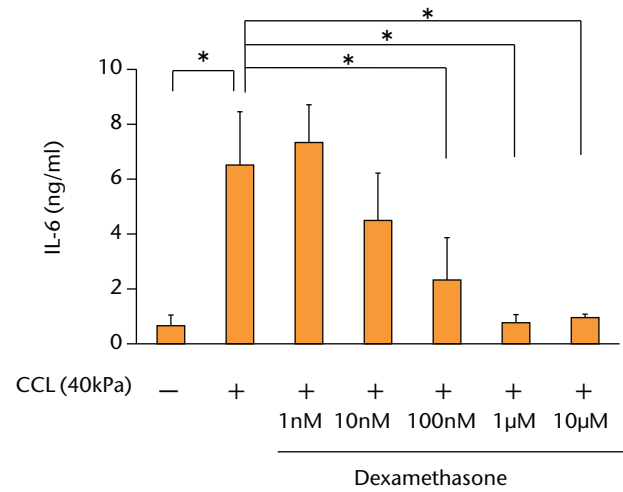


Fig. 4b

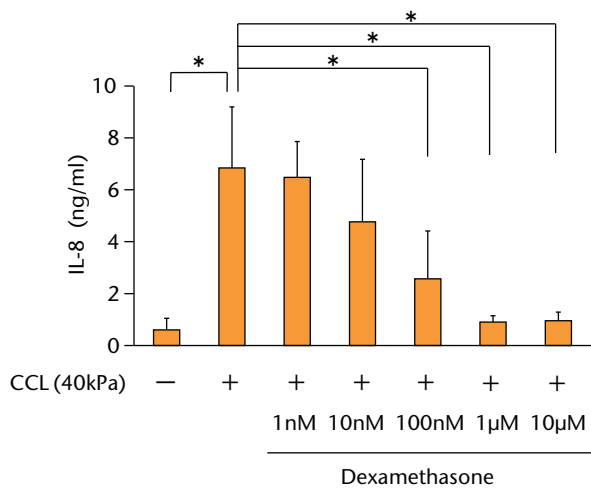


Fig. 4c

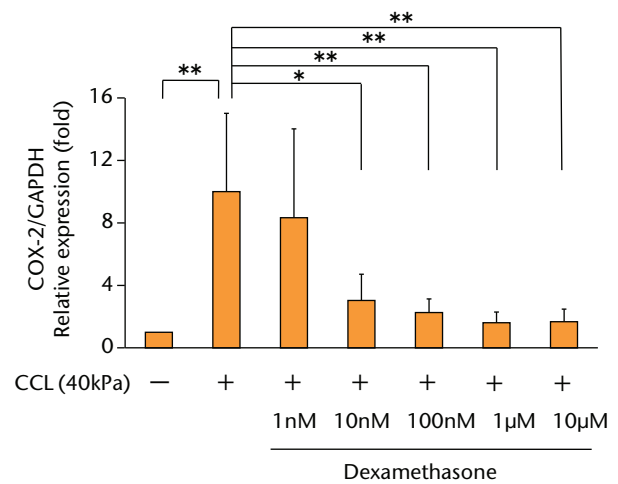


Fig. 4d

Graphs showing the effects of dexamethasone on mechanically induced PGE2, interleukin- (IL)-6, and IL-8 proteins and COX-2 gene expressions. The increased concentrations of a) PGE2 ( $n = 5$ ), b) IL-6 ( $n = 5$ ), and c) IL-8 ( $n = 5$ ) by cyclic compressive loading were suppressed in a dose-dependent manner. d) The upregulation of COX-2 mRNA levels by cyclic compressive loading was also suppressed in a dose-dependent manner ( $n = 5$ ). \* $p < 0.05$  \*\* $p < 0.01$

These functions are still unclear and further studies are required.

Synovial fibroblasts did not produce IL-1 $\beta$  and TNF- $\alpha$  by cyclic compressive loading in this study, as reported previously.<sup>6-10</sup> To our surprise, however, synovial fibroblasts produced PGE2, IL-6 and IL-8 without the stimulation of IL-1 $\beta$  and TNF- $\alpha$ , which are produced by synovial macrophages. Undoubtedly, IL-1 $\beta$  and TNF- $\alpha$ , produced by synovial macrophages, are considered to be key factors for OA development through the production of PGE2, MMPs, and ADAMTSs by synovial fibroblasts and chondrocytes.<sup>2,5,6,15,20,46-50</sup> On the other hand, it has been unclear what triggers the activation of synovial macrophages. In this study, PGE2 was significantly upregulated by cyclic compressive loading without IL-1 $\beta$

and TNF- $\alpha$  stimulation. Also, we have previously demonstrated that cyclic mechanical stress on synovial fibroblasts upregulated mRNA levels of MMP1, MMP2, MMP3, MMP9, MMP13, ADAMTS4 and ADAMTS5 genes in a load-dependent manner through the same experiment.<sup>22,23</sup> Taken together, the upregulation of the key molecules of OA development including PGE2, MMPs, and ADAMTSs was induced by mechanical stress without the upregulation of IL-1 $\beta$  and/or TNF- $\alpha$ . In our opinion, therefore, excessive mechanical stress may 'switch on' these gene expressions as the trigger of OA development without IL-1 $\beta$  and/or TNF- $\alpha$  stimulation. (Fig. 5) This notion may coincide with some previous studies using animals and clinical samples, which showed that IL-1 $\beta$  and/or TNF- $\alpha$  were not necessary in OA development.<sup>51-53</sup>

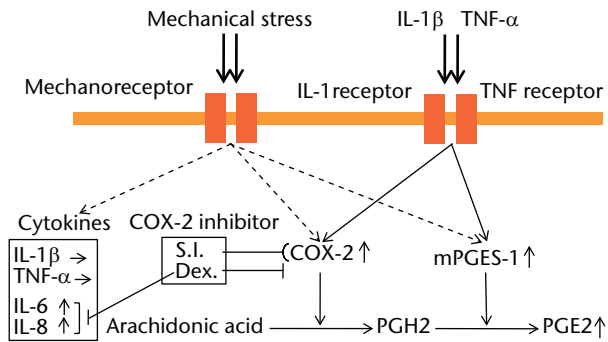


Fig. 5

Schematic representation of the relationship between mechanical stress and the expression of PGE2 and related molecules. Cyclic compressive loading on a 3D cultured construct of human fibroblasts upregulated PGE2, interleukin- (IL)-6 and IL-8 proteins and COX-2, mPGES-1 mRNA levels without IL-1 $\beta$  and tumour necrosis factor- (TNF)- $\alpha$  stimulation. These results indicate that PGE2 upregulation may not be induced via an IL-1 $\beta$  and/or a TNF- $\alpha$  signaling pathway but via other signaling pathways (S.I.; selective inhibitor, Dex; dexamethasone).

IL-1 $\beta$ -deficient mice showed development of OA.<sup>51</sup> Moreover, the recent clinical study showed that IL-1 $\beta$  and TNF- $\alpha$  in the synovial fluid of patients with OA were not significantly higher than that in the control group.<sup>52,53</sup> Therefore, it can be explained that mechanical stress alone is possible to initiate OA development without the stimulation of proinflammatory cytokines.

In a potential limitation of the present study, we did not evaluate other intra-articular cells, such as chondrocytes and meniscal cells. These cells also play an important role in the development of OA. Also, the intracellular signal transductions of PGE2, IL-6 and IL-8 (mechanotransduction) have not been described in detail. In a recent study, mechanotransductions were reported to be related to the Smad pathway,<sup>54,55</sup> mitogen-activated protein kinase pathway,<sup>56-58</sup> or Wnt signaling pathway.<sup>59-61</sup> Our 3D culture system may be useful for the explanation of intracellular mechanotransduction.

In conclusion, cyclic compressive loading on a 3D cultured construct of human fibroblasts upregulated PGE2, IL-6 and IL-8 proteins and COX-2 and mPGES-1 mRNA levels, without IL-1 $\beta$  and TNF- $\alpha$  stimulation. Further investigation may be useful in revealing the molecular mechanism of mechanical stress *in vivo* for a better understanding of the pathology and therapy of OA.

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#### Funding statement:

- This study was supported by a grant from ‘Knowledge Cluster Initiative’ to K. Nakata from The Ministry of Education, Culture, Sports, Science and Technology (MEXT) and Grants-in-Aid for Scientific Research (#23390363 (B)).

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#### ICMJE Conflict of Interest:

- None declared

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