INTRODUCTION:
Prostaglandin E2 (PGE2) plays a significant role in development of osteoarthritis (OA) to cause pain, inflammation, and cartilage degradation (1-3). PGE2 is known to be produced by synovial fibroblasts or chondrocytes in response to proinflammatory cytokines such as IL-1β and/or TNF-α. However, synovitis induced by these proinflammatory cytokines is the secondary pathological event in contrast to the primary excessive mechanical stress on synovial joint in the progression of OA (4). The molecular mechanism of PGE2 production by mechanical stress is still unclear. We hypothesize that human synovial fibroblasts produce PGE2 by mechanical stress via COX-2. To test this hypothesis and further elucidate its signal pathway, we examined the expression of PGE2, IL-1β and TNF-α by cyclic mechanical stress on three-dimensional (3-D) tissue of human synovial fibroblasts with or without COX-2 inhibitor and IL-1β antagonist.

METHODS:
Human synovial fibroblasts were isolated enzymatically from human synovial membranes and the adherent cells were expanded until passage 3 to 7. Collected cells (5.0×10⁵/scaffold) were resuspended in the media and mixed with 1% collagen solution (Atelocollagen®, KOKEN JAPAN). The cell-collagen solution mixture was then seeded onto a collagen scaffold (Atelocell MIGHTY®, KOKEN JAPAN) to produce a 3-D construct. The 3-D constructs were incubated for 3 days before cyclic compressive loading according to our previous methods (5). To examine PGE2 induction by mechanical stress via COX-2 in contrast to IL-1β or TNF-α, cyclic compressive loading of 40kPa (≥10% strain) at 0.5Hz for 1hr, IL-1β (10ng/ml) or TNF-α (100ng/ml) was applied to the constructs with or without the administration of COX-2 selective inhibitor, and then the concentrations of PGE2, IL-1β and TNF-α in supernatant were measured by homogeneous time-resolved fluorescence (HTRF) method after 6 hours. The mRNA expressions of COX-2 and mPGES-1 genes were also examined by quantitative RT-PCR with SYBR Premix Ex Taq (Takara Bio, Shiga, Japan). To further investigate the signal pathway of mechanical stress, cyclic compressive loading (40kPa, 0.5Hz, 1hr) or IL-1β (10ng/ml) was applied to the 3-D constructs with or without IL-1 receptor antagonist (IL-1Ra; Calbiochem, Cat. No.407616), and then the concentration of PGE2 in supernatant was measured with HTRF after 6 hours. Each experiment was repeated more than three times using independent donors. The results of the experiments were analyzed by Mann-Whitney U test and significance was set at p < 0.05.

RESULTS:
The concentration of PGE2 in supernatant was significantly higher in the cyclic loading group as well as IL-1β or TNF-α administration group than in unloaded control group. The concentration of IL-1β or TNF-α was unchanged by cyclic loading (Fig.1). mRNA levels of COX-2 and mPGES-1 genes were also significantly upregulated by cyclic loading (Fig.2). By administering COX-2 selective inhibitor, the increased concentration of PGE2 by cyclic loading was impeded in a dose-dependent manner (Fig.3). By administrating IL-1Ra (1mM), the upregulation of PGE2 by cyclic compressive loading or IL-1β was significantly impeded (Fig.4).

DISCUSSION:
PGE2, COX-2 and mPGES-1 productions were upregulated by cyclic mechanical stress to the constructs, while pro-inflammatory cytokines such as IL-1β and TNF-α remained unchanged. In addition, the administration of IL-1β or TNF-α induced PGE2 production as previously reported in monolayer culture (6). By administering IL-1Ra, the upregulation of PGE2 by cyclic mechanical stress as well as IL-1β was impeded. These results indicate that PGE2 production by cyclic mechanical stress might be induced via IL-1/COX-2 signal pathway without these cytokines (Fig.5).

SIGNIFICANCE:
This study demonstrates the evidence that human synovial fibroblasts directly upregulates PGE2 production by cyclic mechanical stress via COX-2 without IL-1β nor TNF-α, which is one of the possible molecular mechanisms of initiation of osteoarthritis by mechanical stress and would provide better understanding of pathology, therapy, and prevention of development of OA.

REFERENCES:

Fig.1: PGE2 expression by cyclic compressive loading (CCL), IL-1β or TNF-α, and IL-1β and TNF-α expressions by CCL. *; p<0.05

Fig.2: mRNA level of COX-2 and mPGES-1 genes by CCL. *; p<0.05

Fig.3: Inhibition of mechanically-induced PGE2 expression by COX-2 selective inhibitor. *; p<0.05

Fig.4: Inhibition of mechanically or chemically-induced PGE2 expression by IL-1Ra. *; p<0.05

Fig.5: Schematic representation of relationship between mechanical stress and IL-1/COX-2 signal pathway.