INTRODUCTION:
Production of inorganic pyrophosphate (PPi), a natural inhibitor of apatite formation, is an important phenotypic feature of chondrocytes to maintain integrity of articular cartilage. Imbalance of PPi in non-mineralized tissues such as articular cartilage, leads to pathological mineral deposition and change of integrity of cartilage. Once minerals in cartilage are formed and shed into joint spaces, these minerals are capable of inducing inflammatory process and subsequent cartilage degeneration. Elaboration of ePPi by chondrocytes is a bioregulable process, responsive to growth factors and some cytokines. Suppression of chondrocyte ePPi elaboration is reported when cells are cultured with interleukin 1 (IL-1), tumor necrosis factor alpha, procinecendin, IGF-1, and some isoforms of parathyroid hormone related peptide. Bordetella pertussis adenylate cyclase (AC) toxin also suppresses ePPi elaboration by chondrocytes. However, pathogenesis of PPi imbalance is remained unclear, and signal transduction pathway of articular chondrocytes in physiologic or pathologic mineralization has not been fully understood.

IL-6 is one of the major proinflammatory cytokines, and IL-6 and its soluble receptor of IL-6 (IL-6sR) are elevated in synovial fluids from patients with various arthritis, including gout and pseudogout caused by calcium mineral deposition in articular tissues.

We studied the effects of IL-6 and/or IL-6sR on chondrocytes PPi elaboration, vesicle mineralization and examined downstream signal transduction pathways.

METHODS:
Chondrocytes isolation and culture. Chondrocytes were released from the harvested porcine knee articular cartilage by a sequential enzyme digestion method described previously. Cells were plated at high-density monolayer and only used in primary culture to preserve chondrocyte phenotype. Cells were cultured to confluence in DMEM supplemented with 10% FBS and 1% PSF. Growth medium was removed and replaced with serum-depleted medium (DMEM only) the day before initiation of experiments.

IL-6 and/or IL-6sR addition. Culture medium was changed to DMEM, 25 mM HEPES, pH 7.4, 0.5% heat-inactivated FBS, and 1% PSF. IL-6 (0, 10, 50, 100 ng/ml) and/or IL-6sR (0, 50 ng/ml) were added to some cultures. The higher concentrations of IL-6 more than 100 ng/ml have been examined, but plateau effects on ePPi elaboration (data not shown). Aliquots of ambient media and cells were harvested at 48 hours.

PPi Assay. The PPi assay was performed on conditioned media by the 14C-labeled UDPG method as previously described. PPi values were normalized to protein content of cell lysate, as determined by the method of Lowry.

Chondrocyte vesicle biomineralization. Chondrocyte vesicles were isolated from IL-6 and/or IL-6sR-treated chondrocytes. After the exposure to IL-6 and/or IL-6sR for 24 hours, chondrocyte monolayers were washed with DMEM twice, then digested with 0.2% collagenase for 90 min at 37°C. The digest was ultracentrifuged, then at 17,000 x g for 15 min to remove cell debris, then its supernatant was ultracentrifuged at 200,000 x g for 90 min to pellet vesicle fractions. Each vesicle fractions were resuspended in DMEM and protein was assayed by Lowry method. Vesicle fractions were added in pentaplicate in culture. A 30 min incubation. Vesicle fractions were added in pentaplicate in culture. A 30 min incubation.

RESULTS:
IL-6 inhibited PPi elaboration from chondrocytes. IL-6 inhibited PPi elaboration from chondrocytes with increased dose at 48 hours incubation. Additional IL-6sR (50ng/ml) enhanced inhibition of PPi elaboration. IL-1beta inhibited PPi elaboration the most, by 80% at 48 hours. IL-6 alone inhibited PPi by 50%, and with IL-6sR, PPi was inhibited by another 15% (data not shown).

IL-6 increased chondrocytes vesicle mineralization. Vesicle mineralization was increased with IL-6 by 60% and further augmented by addition of IL-6sR (data not shown).

STAT3 is activated during inhibition of PPi elaboration and induced vesicle mineralization. IL-6 dramatically induced STAT3 activation in a dose-dependent manner. The addition of IL-6sR didn't alter STAT3 signals in 15 min. ERK1/2 and Akt were constitutively activated in these chondrocytes and IL-6 and/or IL-6sR did not affect their activation (Figure 1).

DISCUSSION:
Little is known about cellular regulatory mechanisms involved in modulation of chondrocytes PPi elaboration by agonists and antagonists. We showed here that IL-6 inhibited chondrocyte PPi elaboration, increased vesicle mineralization and may lead to loss of chondrocyte specific phenotype and loss of integrity of cartilage. STAT3 was activated during this modulation. Further analysis to confirm the direct STAT3 pathway involvement on inhibition of chondrocyte PPi elaboration and consequent mineral deposition by IL-6 is needed using dominant-negative STAT3 constructs. Understanding pathway for inhibition of PPi elaboration by chondrocytes may elucidate possible therapeutic target in future.