INTRODUCTION
Extensive destruction of collagen fibrils network and loss of the proteoglycan aggrecan are features of articular cartilage degradation in osteoarthritis (OA). Many kinds of enzymes can cleave the matrix molecules during the progression of OA. Recently the role of cathepsin K on cartilage destruction as well as bone resorption was reported. We newly developed antibody to detect specific cleavage site of type II collagen by cathepsin K. Now, this study was designed to investigate the degradation of the cartilage matrix by IL-1, comparing cleavage of type II collagen by cathepsin K to 1) that by collagenases, and 2) to cleavage of aggrecan by aggrecanases as well as MMP using specific antibodies against enzyme-generated neoepitopes.

MATERIALS AND METHODS

**Human articular cartilage explant culture:** This study was approved by an institutional review board and the the patients was given informed consent for the sampling. Articular cartilage was collected from femoral head at the surgery of hip prosthesis for the treatment of femoral neck fracture. The cartilage explants at ~50 mg wet weight per well were cultured in 1 ml of the basic culture medium of DMEM, 1% FBS with or without 2 ng/ml of IL-1β. The medium was changed at day 4, 7 and 11 and cultured up to day 14.

**Antibodies:** We prepared 2 polyclonal antibodies using synthetic peptides of neoepitopes of aggrecan generated by aggrecanase; Glu373-Ala374 (TEGE373) and MMP; Asn341-Phe342 (IPEN341). We also prepared antibodies cleaved neoepitopes of type II collagen by collagenase (COL2-3/4Cshort)(1) and by cathepsin K (C2K)(2).

**Extraction of proteoglycan and cleaved type II collagen:** The cartilages were put in 4 M Guanidium-HCl (GuCl), pH 6.5 with protease inhibitors (1mM EDTA, 1mM Iodoacetamide, and 10 μg/ml Pepstatin A) for overnight. The extracts were dialyzed to PBS and digested the GAG chain by chondroitinase ABC, keratanase I and II. The explants, extracted proteoglycan, were digested by α-chymotripsin to extract the cleaved type II collagen.

**Quantification of GAG:** GAG contents in 4 M GuCl extracts and culture media were determined using a modification of the colorimetric 1,9-dimethylene blue (DMMB) dye assay.

**Western blotting for cleaved neoepitopes of aggrecan by aggrecanase and MMP:** Extracted samples containing 10μg of GAG were loaded per lane. Western blotting were performed to semiquantify the cleaved neoepitopes of aggrecan by aggrecanase (TEGE373) and MMPs(IPEN341) in the explants.

**Immunoassays for specific cleavage sites of type II collagen by collagenase (COL2-3/4Cshort) and cathepsin K (C2K):** Recently we have developed immunoassays to quantify cathepsin K-generated neoepitope as well as collagenase-generated neoepitope. The neoepitopes in the α-chymotripsin-extracts and conditioned medium were determined with these immunoassays.

RESULTS
In IL-1-treated explants, GAG release from the tissues into the media was increased from day 4 to day 14 with IL-1 while content of GAG in the tissues were decreased at day 14(Fig.1).

Fig.1 Quantification of GAG during IL-1-induced cartilage degradation

DISCUSSION
There were significant differences in timing of degradation between aggrecan and collagen based on their specific cleavage sites generated by different degradative enzymes. Interestingly, cleavage of type II collagen by cathepsin K was increased, following cleavage by collagenases. Although mechanisms of induction of cathepsin K by IL-1 are still unclear. There is a possibility that cathepsin K and collagenases could have different mechanisms to be induced and activated by IL-1. Further studies should be needed to study the role of cathepsin K in cartilage degradation during progression of OA. The immunoassay of C2K could be useful to understand the process of matrix degradation in vivo and in vitro.

CONCLUSIONS
IL-1-induced cleavage of type II collagen by cathepsin K was occurred in different timing from cleavage of aggrecan by aggrecanase and MMP and cleavage of type II collagen by collagenase. Cathepsin K could play important role on IL-1-induced cleavage of type II collagen.

REFERENCES