formation), TRACP (osteoclast number), and GAG release (cartilage turnover). Overall cell viability was monitored using the dye Alamar Blue. Passive release from metabolically inactive femur heads was measured as background.

**Results:** Stimulation of the femur heads with RANKL, PTH, IL-1α and OSM + TNF-α led to an increase in CTX-I release. Adding GM6001 to OSM + TNF-α abrogated the release of CTX-I. Femur heads stimulated with IL-1α, OSM + TNF-α and OSM + TNF-α + GM6001 induced an increase in sGAG release. CTX-II release was increased by RANKL, PTH, IL-1α, OSM + TNF-α and IfG-F. CTX-II release from the OSM + TNF-α condition was abrogated when treated with GM6001. The osteoclast marker TRACP increased when stimulated with RANKL, PTH, IL-1α, OSM + TNF-α, OSM + TNF-α + GM6001. PINP release was reduced when stimulating with IL-1α, OSM + TNF-α, OSM + TNF-α + GM6001, whereas PTH and IGF-I increased PINP release.

**Conclusions:** We have established a whole tissue model for osteoarthritis consisting of both cartilage and bone, and which is highly responsive to both catabolic and anabolic stimulation. This is useful for testing potential treatments for OA interfering with more than one aspect of the pathological situation. Further it allows for investigating interactions between cartilage and bone cell types. Hopefully, future treatments for OA may be better identified after the establishment of such a system for drug screening.
inhibited the phosphorylation of p38 and JNK kinases along with its enhancing effect on MKP-1 expression; and when MKP-1 was down-regulated by siRNA, aurothiomalate's ability to inhibit p38 and JNK phosphorylation and COX-2 expression reduced significantly.

Conclusions: The results provide a novel mechanism for the anti-inflammatory action of aurothiomalate through increased MKP-1 expression; and when MKP-1 was down-regulated by siRNA, aurothiomalate's ability to inhibit p38 and JNK phosphorylation and COX-2 expression reduced significantly.

Purpose: Aggrecan, the main proteoglycan of articular cartilage, is composed of a core protein containing 3 globular domains G1, G2 and G3. The interglobular domain between G1 and G2 is the primary site for enzyme attacks of the molecule, matrix metalloproteinases (MMPs) and aggrecanases, generating fragments with specific N-terminal ends. The aim of this study was to identify aggrecan fragments circulating in human serum/plasma with specific antibodies corresponding to neoepitope generated by aggrecanases.

Methods: Pools of serum from controls (n = 22; mean age: 62 yrs ± 2.7) and patients suffering from osteoarthritis (n = 19; mean age: 64: ±4) and plasma from patients suffering from RA (n = 20; 10 men and 10 women, mean age: 61.7 yrs ±3.9, DAS >4.8) were investigated with affinity purification and Western blots using two monoclonal antibodies. First, monoclonal antibody BC-3 recognizing a neoepitope generated by aggrecanases in position 374-ARGSV. Secondly, a monoclonal antibody F-78 was used recognizing a repetitive epitope exposed at least twice on G1 and G2 domains.

Results: In Western blot, BC-3 recognized a band migrating at 45kDa in RA patients only. The strong reactivity was abolished by pre-incubation with the peptide corresponding to BC-3 epitope. To further investigate the distribution of ARGSV-carrying neoepitope in RA plasma, we subjected the plasma to affinity purification and Western blots using two monoclonal antibodies. First, monoclonal antibody BC-3 recognizing a neoepitope generated by aggrecanases in position 374-ARGSV and containing G2 domain suggesting that this fragment is a good candidate marker for a sandwich assay using monoclonal antibodies BC-3 and F-78. Apparently, this fragment of aggrecan is primarily found in circulation of RA patients and additional investigations are underway to determine the molecular nature of this fragment.

Purpose: One hallmark of the progression of osteoarthritis and rheumatoid arthritis is the gradual destruction of articular cartilage in affected joints, in part mediated by the matrix metalloproteinases (MMPs) and aggrecanases. Collagen type II is found almost exclusively in cartilage tissue where it constitutes over 60% of the dry weight. Degradation of collagen type II is for the major part mediated by MMPs. Because of the abundance of collagen type II in cartilage and its tissue specificity, MMP generated fragments of type II collagen (neo-epitopes) are highly interesting as cartilage degradation markers. The aim of this study was to develop new biochemical enzyme immuno assays (EIA) of MMP cleaved collagen type II fragments to be used as markers of cartilage degradation.

Methods: Two neoepitopic sites representing ubiquitous MMP-generated fragments of collagen type II identified from cleavage of collagen type II were selected. The two neoepitopes corresponded to cleavage between amino acids 845–846 (neoepitope I) and 872–873 (neoepitope II). Peptides reflecting the neoepitopic sites (845–846 and 872–873) were synthesized and used for immunization of balb/C mice and rabbits. Competitive EIA assays were established using biotinylated synthetic peptide as coater and the immune sera as primary antibody. The assays were used to profile the proteolytic events in the well characterized ex vivo articular cartilage explant model. The cartilage explants included catabolically stimulated TNFα (tumor necrosis factor alpha) and oncostatin M cultures, non-stimulated cultures, and metabolically inactive cultures.

Results: In catabolically stimulated bovine cartilage explant cultures a low concentration of neoepitope I of below 50 ng/ml was found in samples collected day 3, 6, and day 11. However after prolonged catabolic stimulation of 22 days a more than 20-fold dramatic increase was observed reaching a level higher than 1000 ng/ml. In comparison the control cultures of non-stimulated explant culture media had the same low level below 50 ng/ml on day 3, 6, and 11, and reached only 100 ng/ml at day 22. The metabolically inactive culture remained below 50 ng/ml during the complete culture period. A similar pattern of a dramatic increase in the 22-day catabolically stimulated culture was observed in human articular cartilage explant cultures. The pattern of other marker of neoepitope II mirrored that of neoepitope I.

Conclusions: Two new immunoassays of neoepitope markers of collagen type II degradation were established. Measurement of these neoepitopes in both the bovine and human ex vivo articular cartilage explant model demonstrated that these markers reflect the cartilage degradation. Further studies are needed to investigate whether these fragments are of clinical relevance.