Methods: Human cartilage explants were cultured in the presence or absence of the catabolic cytokines oncostatin M (OSM) and tumor necrosis factor alpha (TNFα). Fragments originating from aggrecanase and MMP-mediated cleavage of aggrecan and type II collagen and released into the supernatant was determined using a range of neo-epitope specific immunoassays; (1) sandwich ^{342}FFGVG-G2 ELISA, (2) competition NITEGE^{373}ELISA (3) sandwich G1-NITEGE^{373} ELISA (4) competition ^{374}ARGSV ELISA, and (5) sandwich ^{374}ARGSV-G2 ELISA all detecting aggrecan fragments, and (6) sandwich CTX-II ELISA, which is specific for the cross-linked neo-epitope EKGPDPxEKGPDP originating from the C-telopeptide of type II collagen.
Results: We found that quantitatively the accumulated release of aggrecanase- and MMP-derived aggrecan fragments was similar in bovine and human cultures of articular cartilage upon stimulation with catabolic cytoikines. However, in human cultures these fragments were continuously released during all phases of the 21 day culturing period, whereas in bovine cultures MMP-derived fragments were only observed in the late phase (day 16-21) and aggrecanase-derived fragments in the early and mid phase.
Conclusions: Our data confirm that major differences in bovine and human processing of articular cartilage exist in response to catabolic cytokines. This suggest that careful consideration should given to the application of this ex vivo model in drug screening programmes.

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SUPPRESSION OF MMP ACTIVITY IN BOVINE CARTILAGE EXPLANTS CULTURES HAS LITTLE IF ANY EFFECT ON THE RELEASE OF AGGRECAN FRAGMENTS CARRYING AGGRECANASE-DERIVED NEO-EPITOPES, WHEREAS CORRESPONDING MMP-DERIVED AGGRECAN AND COLLAGEN FRAGMENTS ARE COMPLETELY ABOLISHED FROM THE SUPERNATANT

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Purpose: Progressive loss of articular cartilage is a central hallmark in many joint disease, however, the relative importance of individual proteolytic pathways leading to cartilage erosion is at present unknown. In particular, the metabolic response induced by the inhibition of MMPs remains to be investigated in appropriate model systems, which also includes assessment of aggrecanase-derived matrix degradation. We therefore investigated the time-dependant release *ex vivo* of MMP- and aggrecanase-derived fragments of aggrecan and type II collagen into the supernatant of bovine cartilage explants cultures using neo-epitope specific immunoassays, and to associate the release of these fragments with the activity of proteolytic enzymes using inhibitors.
Methods: Bovine cartilage explants were cultured in the presence or absence of the catabolic cytokines oncostatin M (OSM) and tumor necrosis factor alpha (TNFα). In parallel, explants were co-cultured with protease inhibitors such as GM6001, TIMP1, TIMP2 and TIMP3. Fragments originating from aggrecanase and MMP-mediated cleavage of aggrecan and type II collagen and released into the supernatant was determined using a range of neo-epitope specific immunoassays; (1) sandwich ^{342}FFGVG-G2 ELISA, (2) competition NITEGE^{373}ELISA (3) sandwich G1-NITEGE^{373} ELISA (4) competition ^{374}ARGSV ELISA, and (5) sandwich ^{374}ARGSV-G2 ELISA all detecting aggrecan fragments, and (6) sandwich CTX-II ELISA, which is specific for the cross-linked neo-epitope EKGPDPxEKGPDP originating from the C-telopeptide of type II collagen.
Results: We found that (1) aggrecanase-derived aggrecan fragments are released in the early (day 2-7) and mid phase (day 9-14) into the supernatant from bovine explants cultures stimulated with catabolic cytokines, (2) the release of NITEGE^{373} neo-epitopes are delayed compared to the corresponding ^{374}ARGSV fragments, (3) the MMP inhibitor GM6001 did not reduce the release of aggrecanase-derived fragment, but induced a further delay in the release of these fragments, (4) no significant differences between aggrecan profile obtained with competitive assays vs corresponding sandwich assays could be detected, finally (5) the MMP-derived aggrecan and type II collagen fragments were released in the late phase (day 16-21) only.
Conclusions: Our data support the model, that aggrecanases and MMPs act independently in the processing of the aggrecan molecules, and furthermore that suppression of MMP-activity had little if any effect on the quantity of aggrecanase-derived fragments released from explants cultures.

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BIOMECHANICAL PROPERTIES OF THE BONE AND CARTILAGE COMPARTMENTS IN A RAT KNEE OA MODEL

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Purpose: Osteoarthritis (OA) is a disease that affects the entire articular joint, with both changes to the bone and cartilage compartments.
Optimal biomechanical properties are pivotal for maintenance of joint integrity and function. Altered biomechanical properties of bone and cartilage are observed in OA disease and might add to worsening of disease.
We investigated biomechanical properties of the bone and cartilage compartments in healthy and affected rat knees from an OA model with combined traumatic knee surgery and increased bone turnover.
Methods: Two groups each of 10 6-months old rats were subjected to a combined ovariectomy and partial medial meniscectomy (OVX+MNX) or sham surgery (SHAM).
Animals were sacrificed 8 weeks after surgery, and one tibia was processed for biomechanical indentation testing. The tibias were cast in Acrylfix-mould with the shaft fixated in the mould and the tibial plateau protruding.
Indentation test was performed centrally on the medial part of the tibial plateau (Figure 1). An indenter area of 0.78 mm^2 and a fixed

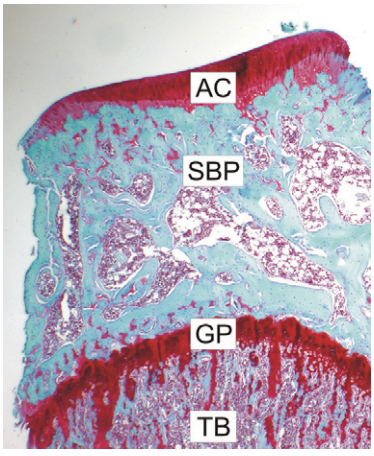

Figure 1. AC: Articular cartilage; SBP: Subchondral bone plate; GP: Growth plate; TB: Trabecular bone.