Results: Coculture of the U937 cells with synovial fibroblasts resulted in differentiation of U937 into monocytes, as evidenced by FACS indicating that they were CD14+. Over 7 days, the cytokine and chemokine levels in the media of these cocultured cells with cartilage explants approached levels similar to those reported for OA synovial fluid, including low levels of Il-1β and TNFα. GAG release was observed in co-cultures stimulated with Il-1β, uPA, or Fn-f, with the most severe effects in the Il-1β-treated group (Figure). However, the concentration of Il-1β used in the control samples, typical of concentrations used by others for release of GAGs from cartilage disks in vitro, is far greater than the concentration reported in OA synovial fluid. Pretreatment with Il-1ra was able to inhibit the loss of GAG from the cartilage in the Il-1β stimulated samples, but did not block Fn-f-stimulated GAG release in the co-culture system, and had a small effect on uPA stimulated GAG release.
Conclusions: An in vitro model of cartilage degradation has been developed utilizing a co-culture of human synovial fibroblasts with differentiated U937 cells, in the presence of bovine cartilage explants. The interaction of these cell types generated a cytokine profile similar to OA synovial fluid, and stimulation of this system with uPA or Fn-f led to cartilage degradation (GAG loss). Our ongoing work will continue to explore the roles of various cytokines and the molecular mechanisms through which they influence cartilage degeneration in this in vitro joint model system, and their potential relation to OA disease progression.

Matrix Biochemistry

444 OSTEOARTHRITIS AND AGE RELATED CHANGES IN AGGRECAN AND COLAGEN IN HUMAN HIP CARTILAGE

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Purpose: To confirm that previously reported changes in collagen and GAG content from knee OA cartialge also occur in hip OA and that these changes are different from those in reference cartilages. We also examined the relationships between total collagen, % denatured collagen and GAG content and with age.
Methods: 18 OA (aged 45 to 81, 11 women) and 26 reference (aged 55 to 99 years, 20 women) femoral heads were collected at hip replacement surgery, due to OA or femoral neck fracture. OA cartilages were taken from sites where it was possible to sample full-depth cartilages whereas reference cartilages were taken from the superior most loaded areas. Cartilages from the same site were examined for routine histology and immunohistochemistry. Each sample was divided into two parts that were either, digested with 1 mg/ml α-Chymotrypsin in 50 mM tris containing the proteinase inhibitors to extract denatured collagen, or with papain at 60C for 18 h (1−2 μl of papain/10 mg wet weight tissue) in 0.1 M tris-HCl, pH 7.2, containing 0.01 M disodium EDTA and 0.005 cysteine-HCl to enable GAG analysis. The Chymotrypsin supernatants (containing cleaved collagen) and residues (containing intact collagen) were hydrolysed in 12N and 6N HCl, respectively, at 110ºC overnight and then dried. Subsequently the samples were dissolved in 500 μl of distilled water and clarified using an equal amount of charcoal and AG-1 X8 anoin exchange resin. The amount of hydroxyproline (Hyp. μg/mg wet weight) was measured colorimetrically at 550 nm. The values from the assay were compared to those from a standard curve prepared with L-4-Hydroxyproline. Amounts of hydroxyproline were expressed either as total collagen or % degradation. The papain-digested samples were assayed for GAG by a commercially available kit using dye-precipitation of sulphated GAGs with Alcian blue (www.wieslab.com).
Results: The reference group was older than the OA group ($p < 0.001$). Collagen content was 23% lower in OA than reference cartilage ($p = 0.002$). % Denatured collagen was 2.5 times higher in OA than that of reference cartilages ($p = 0.001$).
% Denaturation was inversely related to collagen content in OA cartilage ($r = -0.78$, $p = 0.000$) but not in the reference cartilage ($p = 0.5$).
GAG content was similar in reference and OA cartilages. GAG content was inversely related to % denatured collagen in OA and reference samples; lower GAG was present in samples with more denatured collagen. Amounts of collagen and GAG and % denatured collagen were not related to patients' age. The Mankin and immunohistochemistry grades, higher in OA than in reference samples ($p < 0.001$), were not related to % denaturation, collagen or GAG content.
Conclusions: The present study provides new perspectives of the relationships between the two main molecules, collagen and aggrecan, in OA and reference articular cartilage. Similar collagen damage in hip OA cartilage as previously shown in knee OA cartilage suggest similar OA degradative mechanisms of collagen in different joints. With respect to age, our study shows similarities between OA and reference cartilage. The lack of relationships between molecule contents and age may suggest that subjects age is not related to disease activity or suseptibility. Rather we believe that exposure time to a risk factor is of importance. The increased degree of denatured collagen and the inverse relationship between % denatured collagen and collagen content in OA compared to reference cartilage indicates mechanisms of progressiveness in disease that are not activated in normal ageing. Therapeutic interventions in progressive hip and knee OA may include means to control cartilage collagen content using inhibitors that decrease collagen degradation as well as treatments that increase cartilage GAG content in order to improve cartilage quality.

445 P38 MAPK IS AN ESSENTIAL UPSTREAM SIGNALLING PATHWAY IN PROTEOLYTIC CARTILAGE DEGRADATION

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Purpose: Matrix Metalloproteases (MMP) and aggrecanases are essential players in cartilage degradation. However, the signaling pathways involved that results in MMP and aggrecanase synthesis and activation are not well understood. We investigated whether the p38 MAPK was involved in processes leading to collagen type II degradation in catabolic stimulated articular cartilage.
Methods: Bovine articular cartilage explants were isolated from stifle joints from slaughtered cows 1.5−2 years of age. The explants were cultured serum-free in Dulbecco's Modified Eagle's Medium in the presence of 10 ng/ml Oncostatin M (OSM) and 20 ng/ml Tumor Necrosis Factor-α (TNF) with or without the specific inhibitors of p38 MAPK SB-203580 or SB-202190, respectively, in doses of 0.1, 1 or 10 μM for 21 days with change of conditioned medium every 2−3 day. Cartilage explants cultured in medium alone and metabolically inactivated explants were used as control. Cell viability was followed by the Alamar blue colorimetric assay. Total cartilage proteoglycans were isolated from the cultured cartilage explants after the culture period by 4 M GuHCl extraction for 48 h. Collagen type II degradation was analyzed by the CTX-II assay and by hydroxyproline quantification. MMP activity was assessed in the conditioned medium using the Mca-Pro-Leu-Gly-Leu-Dap(Dnp)-Ala-Arg-NH_2 MMP substrate with a quencher and a highly fluorescent end, and the cleavage was followed by quantification of the fluorescence.
Results: Stimulation of cartilage degradation by OSM + TNF resulted in a 100-fold induction of CTX-II release ($p < 0.01$), compared to vehicle treated and metabolic inactive controls. The total levels of proteoglycan in the explants decreased by 50%, $p < 0.0001$ in the OSM + TNF treated cartilage compared to vehicle control, but the p38 inhibitor SB-203580 was able to retain the levels of proteoglycans by 35%, $p < 0.01$, compared to vehicle control. Exposure of the articular cartilage explants to OSM + TNF, and the specific p38 MAPK inhibitors SB-203580 or SB-202190, resulted in a dose-dependent complete abrogation of MMP mediated collagen type II degradation (CTX-II). This was in alignment to the quantification of hydroxyproline levels, ($p < 0.01$). In support, p38 MAPK inhibition resulted in a dose-dependent abrogation of MMP activity measured by the fluorescence assay. In addition, no toxic effect was observed on cell viability.
Conclusions: We found that the inhibition of p38 MAPK abrogated proteolytic cartilage degradation by blocking MMP synthesis and activity. These results suggest that pathways resulting in less p38 signaling may be potential options for indirect modulation of MMP expression and activity. This may be important for the discovery of new potential OA treatments.

446 DETECTION OF THE EARLY CHANGE OF THE CARTILAGE DEGENERATION WITH FTIR AND ULTRASOUND EVALUATION

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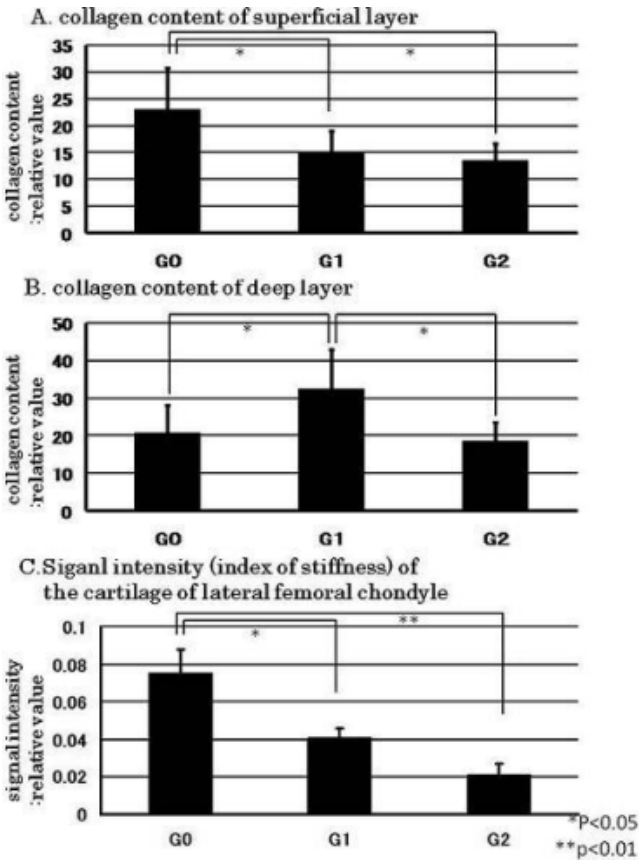
Purpose: Early diagnosis of osteoarthritis is important, although the diagnostic method is not fully established yet. It is well known that

collagen content decreases in late stage osteoarthritis. In early stage, it is reported that anabolism of the collagen increases. The hypothesis of this study is that the collagen content increases in the slightly degenerated cartilage. In our group, material properties of the articular cartilage were examined with ultrasound cartilage evaluation system. In this study, Fourier Transform Infrared (FTIR) imaging was also used to evaluate the collagen content of articular cartilage.

Methods: Fourier Transform Infrared spectrometer (FT-IR-460 PLUS: JASCO: Tokyo) coupled to a microscope (Intron-IRT-30: JASCO: Tokyo) was used to acquire data. Human articular cartilage was obtained at total knee arthroplasty and was graded by ICRS grading. Then, the mechanical properties of the cartilage were measured with our ultrasound cartilage evaluating system with wavelet transform method. Three parameters were used; signal intensity (index of the stiffness), signal duration (index of roughness) and signal interval (index of thickness). Then frozen section of 5 μm was made for FTIR imaging. In FTIR imaging, the integrated area under the protein amide I and II (1710–1570 cm^{-1}) was used as collagen content. For quantitative analysis, the cartilage of weight bearing area of the lateral femoral condyle was selected to avoid the site dependent variance. Sample number was 10 of Grade-0 (G0), 10 of G1, 6 of G2 and 0 of G3 from 10 patients. In statistical analysis, one-way ANOVA was used and $p < 0.05$ was defined to be significant.

Results: In the mapping of collagen content with FTRI, in G0 cartilage, collagen rich area existed in surface and deep layer. In G1 cartilage, the collagen content of superficial area decreased and the collagen content in deep layer increased. In G3, collagen content decreased in whole layer. In quantitative analysis, there was no difference in collagen content between G0, G1 and G2 cartilage when whole layer of cartilage was examined. In superficial layer, collagen content decreased as ICRS advanced (Figure A). Otherwise, in deep layer, the G1 cartilage had significantly richer collagen content than G0 or G2 cartilage (Figure B). In ultrasound evaluation, signal intensity declined (Figure C) and signal duration increased as ICRS grade advanced. There was significant difference between G0 and G1 cartilage in these two parameters. Further examination whether the signal intensity (index of the cartilage stiffness) reflects the collagen content of the cartilage was performed. Signal intensity had a significant correlation with collagen content in superficial layer ($R = 0.80$).



Conclusions: In this study, the change of the collagen content in G1 cartilage differs in superficial layer and in deep layer. As a result of an increase of anabolism, the collagen content of G1 cartilage increased in deep layer. However, collagen content of superficial layer decreased. We think that catabolism occurs stronger than anabolism and the collagen content decreases. Recently, the collagen content of the cartilage has been measured non-invasively with such as MRI T2 mapping method. These changes of the collagen content of G1 cartilage may become a candidate for early diagnosis of the cartilage degeneration. Ultrasound is also a non-invasive method and the change of the signal intensity can be used to guess the collagen content of the superficial layer of articular cartilage.

447 MATRIX METALLOPROTEINASE (MMP)-12 REGULATES MMP-9 EXPRESSION IN INTERLEUKIN-1β-TREATED ARTICULAR CHONDROCYTES

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Purpose: Limited information is currently available on the expression levels and roles of matrix metalloproteinase (MMP)-12 in chondrocytes. In this study, we characterize the expression mechanisms of MMP-12 and its possible function in cartilage destruction.

Methods: The expression mechanisms of MMP-12 and MMP-9 were determined using reverse transcriptase polymer chain reaction (RT-PCR), quantitative real-time polymerase chain reaction (qRT-PCR), Western blotting, reporter gene assay, and zymography in primary culture chondrocytes either treated with interleukin (IL)-1β or left untreated. The roles of mitogen-activated protein (MAP) kinase subtypes were additionally examined with specific inhibitors. The function of MMP-12 was assayed in chondrocytes treated with the recombinant active protein.

Results: Il-1β induced the expression, secretion and activation of MMP-12 in chondrocytes and cartilage explants via MAP kinase pathways. Extracellular signal-regulated kinase and p38 kinase regulated MMP-12 expression, whereas c-jun N-terminal kinase modulated activation. MMP-12 enhanced MMP-9 expression and secretion in the presence of Il-1β in chondrocytes.

Conclusions: Il-1β in chondrocytes induces the expression and activation of MMP-12, which, in turn, augments MMP-9 expression and secretion. Our results support the possible involvement of MMP-12 in cartilage destruction.

Mechanobiology

448 RHEOLOGICAL PROPERTIES OF OSTEOARTHRITIC SYNOVIAL FLUID BEFORE AND AFTER ADDITION OF DIFFERENT HYALURONIC ACID FORMULATIONS

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Purpose: To assess in vitro the effect of 2 formulations of viscosupplement, linear and a cross-linked hyaluronic acid (HA) on the synovial fluid (SF) rheology.

Methods: SF were obtained by sterile aspiration of the affected joints in 14 patients suffering from knee OA. SF samples were collected in sterile tubes and stocked at 4ºC. SF were characterized by size exclusion chromatography (SEC) and rheology. From SEC experiments it was possible to determine the different parameters (concentrations, average molecular weights of HA). Two viscosupplements (a cross-linked HA with an apparent MW of 6 mDa and a linear HA with an average MW of 1.1 mDa) were then added to SF in a ratio 1/1 to test their effects on SF behaviour. The rheological behaviour of SF and SF+HA was determined using an AR 1000 rheometer.

Results: The concentration of HA varied from 0.2 to 2.5 g/L. In steady state flow the rheological behaviour of SF was non Newtonian. The addition of linear HA modified only slightly the behaviour of SF. It especially increased viscosity at higher shear rate and decreased shear rate dependence of the SF suggesteing the SF/exogenous HA interaction. On the opposite, the addition of cross-linked HA gave a large non-Newtonian