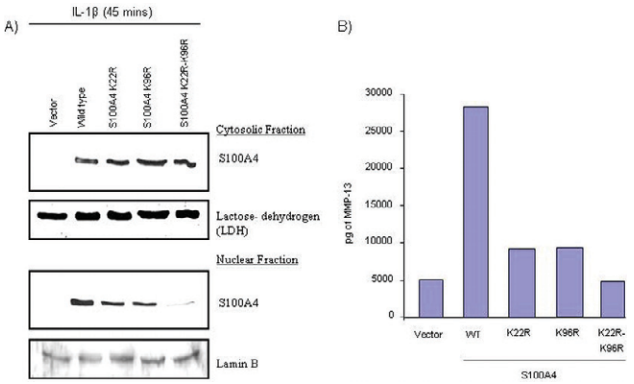

Figure 1

region of MMP-13 and modulates its expression. Thus, our study provides a novel pathway for IL-1β mediated production of MMP-13 in chondrocytes. Since both S100A4 and IL-1β have been found to be increased in OA cartilage, this novel pathway could contribute to cartilage degradation in OA.

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THE ASSOCIATION OF CHONDROCYTE MECHANICAL PROPERTIES AND PHENOTYPIC EXPRESSION DURING CELLULAR EXPANSION ON MICROPATTERNED SUBSTRATES

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Purpose: Chondrocytes undergo de-differentiation as a result of *in vitro* cell expansion, exhibiting a more fibroblastic phenotype with serial passage in monolayer. The differentiation state of these cells has been characterized using phenotypic biomarkers such as gene and protein expression, cell surface receptors, and cell morphology. Using micropatterned substrates to control cell shape, we examined the hypothesis that the biomechanical properties of chondrocytes are altered in association with de-differentiation during passage.
Methods: Superficial and middle/deep zone chondrocytes were isolated from knee cartilage from skeletally mature pigs (N = 10). Chondrocytes were serially expanded for three passages (P0-P3), and single-cell viscoelastic properties were measured after each passage (n = 43-58) using atomic force microscopy (AFM). Mechanical tests were also measured for passaged cells cultured on restrictive micropatterned surfaces (8 μm islands) created using self-assembled monolayers (SAMs) (n = 21-31, Fig. 1). The differentiation state of zonal chondrocytes was verified using quantitative real-time PCR of collagen I and II.
Results: Characteristic differences between superficial and middle/deep zone cells were present at P0 as evidenced by their mechanical properties and gene expressions. After subsequent monolayer expansion, cells progressed towards a more homogeneous mechanical and biochemical phenotype. Subsequent culture on micropatterned surfaces appeared to induce a recovery of cellular mechanical characteristics towards the chondrocytic phenotype.
Conclusions: The current findings support the hypothesis that cellular biomechanical properties correlate with phenotype. Results suggest that chondrocyte de-differentiation is characterized by phenotypic changes in both biochemical and biomechanical characteristics. The effects of de-differentiation on chondrocytes can be partially ameliorated by maintaining a spherical cell morphology using a micropatterned SAM. These findings demonstrate that patterned self-assembled monolayers can provide a novel method for investigating the relationship between cell shape and phenotypic expression.

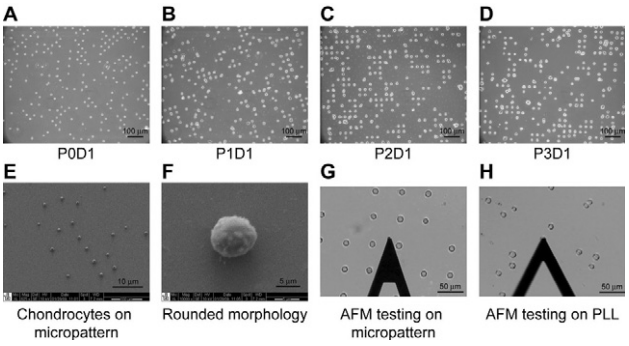

Figure 1. Zonal chondrocytes were seeded onto micropatterned SAM surfaces after each passage, P0D1-P3D1, and cultured for one day before analysis (A-D). SEM images of the patterned cells indicated regular spacing (E) and a rounded morphology (F). Elastic and viscoelastic properties were measured via AFM for superficial and middle/deep zone cells on micropatterned (G) and non-patterned, poly-L-lysine-coated (PLL) surfaces (H).

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GREEN TEA POLYPHENOL EPIGALLOCATECHIN-3-GALLATE (EGCG) INHIBITS ADVANCED GLYCATION END PRODUCTS-INDUCED EXPRESSION OF TUMOR NECROSIS FACTOR-α AND MATRIX METALLOPROTEINASE-13 IN HUMAN OSTEOARTHRITIS CHONDROCYTES

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Purpose: Osteoarthritis (OA) is the most common disease in the aging population. Etiology of OA is unknown and the mechanisms associated with OA pathogenesis are only partly understood. Age-related accumulation of advanced glycation end products (AGEs) have been shown to activate human chondrocytes resulting in the induction and production of proinflammatory cytokines and matrix metalloproteinases (MMPs). Tea is the most widely consumed beverage in the world and possesses human health benefits. In the present study we examined the effect of EGCG on AGE-BSA-induced activation of NF-κB and production of TNF-α and MMP-13 in human OA chondrocytes.
Methods: The macroscopic cartilage degeneration was determined by staining of femoral head samples with India ink and the cartilage with smooth articular surface ("unaffected cartilage") was resected and used to prepare chondrocytes by enzymatic digestion. OA chondrocytes were cultured at high density in monolayer. Expression of chondrocyte-specific genes was determined by RT-PCR. AGE-BSA was prepared by reacting endotoxin-free BSA with glycoaldehyde. Characterization of AGE-BSA was performed spectrophotometrically at 340 nm and AGE-specific fluorescence was detected at excitation/emission wavelengths of 360/430 nm, 330/395 nm, 365/440 nm, 485/530 nm, 280/350 nm and band widths set at ex40/em40. Electrophoretic migration of native and modified BSA samples was analyzed by reducing SDS-PAGE. Cytotoxicity of AGE-BSA and EGCG was examined by using a Cytotoxicity Assay Kit. OA chondrocytes were pretreated for 2 h with different doses of EGCG (25-200 μM) and then stimulated with AGE-BSA (600 μg/ml) or native BSA. Gene expression of TNF-α and MMP-13 was determined by TaqMan assay. Production of TNF-α was determined using cytokine-specific ELISA. Western immunoblotting was used to analyze the MMP-13 production in the culture medium and the enzyme activity was assayed

by gelatin zymography. Phosphorylation of p38 and JNK MAPKs, and the activation of nuclear factor (NF)-κB was determined by Western immunoblotting. DNA binding activity of NF-κB p65 was determined using an ELISA. IκB kinase (IKK) activity was determined using *in vitro* kinase activity assay. Statistical analyses were performed using Origin 6.1 software package and $P < 0.05$ was considered significant.

Results: Characterization of AGE-specific modifications of glycoaldehyde treated BSA is shown in Figure 1. We found that primary or passage 1 human OA chondrocytes in monolayer culture expressed COL2A1, Aggrecan and SOX-9 mRNAs, whereas COL10A1mRNA was not detected. EGCG and AGE-BSA were not cytotoxic at the doses used. Our results showed that EGCG significantly decreased AGE-BSA-stimulated gene expression and production of TNF-α and MMP-13 in human OA chondrocytes. The inhibitory effect of EGCG on the AGE-BSA induced expression of TNF-α and MMP-13 was mediated at least in part via suppression of p38-MAPK and JNK activation. In addition, EGCG inhibited the phosphorylating activity of IKKβ kinase in an in vitro activity assay. EGCG also inhibited the AGE-BSA-mediated activation and DNA binding activity of NF-κB by suppressing the degradation of IκBα resulting in its accumulation in the cytoplasm.

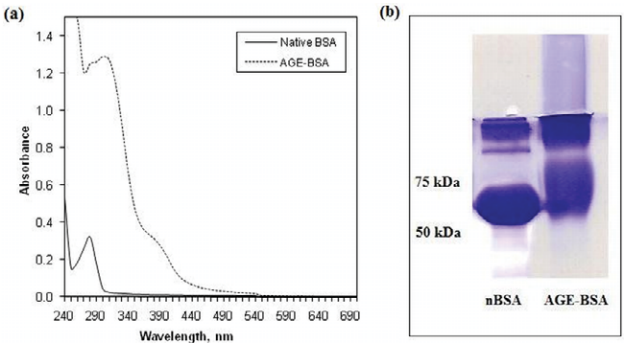

Figure 1

Conclusions: These novel pharmacological actions of EGCG on AGE-BSA stimulated human OA chondrocytes provide new suggestion that EGCG or compounds derived from it may inhibit cartilage degradation by suppressing AGEs-mediated catabolic response in human OA chondrocytes.

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WOUNDING OF ARTICULAR CARTILAGE OR SYNOVIUM ACTIVATES INTRACELLULAR SIGNALLING INCLUDING NUCELAR FACTOR KAPPA B AND SRC

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Purpose: Injury to joints predisposes to osteoarthritis. The molecular response of connective tissues to injury, and how this leads to degeneration is not understood. Our group has previously shown that sharp injury to cartilage (either dissection or scoring of the intact articular surface) rapidly activates the mitogen-activated protein kinases (MAPKs). These pathways are typically activated by inflammatory stimuli such as IL-1, but no such soluble mediator appears to be released from injured cartilage. I set out to examine a) whether other intracellular signalling pathways such as NFκB and tyrosine kinases were activated by cartilage injury, b) if the signalling was sufficient to induce inflammatory response genes and c) whether this response was particular to cartilage, or seen in other connective tissues such as synovium too.

Methods: Tissue for experiments was dissected from porcine metacarpophalangeal joints. Some joints were injected with 10μM PP2 prior to dissection and culture of cartilage in serum-free medium. Tissue lysates were assayed for phospho-kinases by western blotting. IκB kinase (IKK) activity in lysates was assayed by immunoprecipitation-kinase assay, by measuring phosphorylation of the substrate IκBα. Phosphotyrosine immunoprecipitation and western blotting was carried out with 4G10 antibody. Tyrosine-phosphorylated proteins were eluted with phenyl phosphate, electrophoresed, and silver-stained bands identified by mass spectrometry. Cartilage sections were stained with a fluorescently-labelled antibody to p65 and examined by confocal microscopy. RNA was extracted from cartilage explants and mRNA levels of inflammatory response genes assessed by RT-PCR.

Results: Nuclear translocation of the p65 subunit of NFκB was evident 30 minutes after cartilage injury. IKK activity was rapidly induced following dissection and was not dependent on exposure to culture medium. A number of mRNAs including ADAMTS-4, MMP-1 and COX-2 were induced within 4 hours of injury. Certain tyrosine phosphorylations were induced within seconds of cartilage dissection. 3 out of the 4 main injury-regulated, tyrosine-phosphorylated proteins were purified and identified by mass spectrometry as focal adhesion kinase, paxillin and cortactin. These were all known substrates of src kinase. Prior injection of the specific src inhibitor PP2 markedly reduced injury-regulated tyrosine phosphorylation, suggesting that this response was src-dependent. However, PP2 did not affect the induction of MAPKs or IKK following cartilage injury. MAPK and IKK activation was also seen following dissection and culture of synovium. The phosphotyrosine signature of injured synovial lysates was strikingly similar to that of injured cartilage. Phosphorylation of the same 3 src substrates was again seen following injury to synovium.

Conclusions: A wide range of intracellular signalling is activated in parallel following either cartilage or synovial injury, including MAPKs, NFκB and src-dependent signalling. It would seem likely that this represents a conserved response of connective tissues to injury. The signalling is sufficient to induce inflammatory response genes. The mechanism(s) of activation of NFκB and src signalling after injury is unknown, but is likely to be physiologically relevant to the tissue, and may provide a link between injury and matrix degradation.

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DEATH OF CHONDROCYTES IN MURINE ARTICULAR CARTILAGE: EFFECT OF AGE

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Purpose: Although aging-related chondrocyte death in articular cartilage has been reported, it has not been consistently observed and has not been well studied in mice, a species increasingly being utilized for OA research. The purpose of the present study was to determine the relationship between the extent of chondrocyte cell death and age in the proximal tibiae of mice.

Methods: Control (unoperated) stifle joints from 5 different groups of C57/BL6 mice (93 total), aged 18 weeks (n=11), 22 weeks (n=13), 16 months (n=10), 17 months (n=33), or 23 months old (n=26), were evaluated (Study 1). One joint/mouse was routinely fixed, decalcified, processed, embedded intact into paraffin, and serially sectioned in a coronal plane. One representative midcoronal section from each joint was selected for evaluation and was routinely stained with hematoxylin & eosin. Total area of chondrocyte cell death (CCD) within the articular cartilage in each tibial plateau was measured by tracing areas where 2 or more chondrocytes were dead, as determined by loss of basophilia of nuclei