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bilization compared to the control group by ISH and qPCR. The expression was not changed after immobilization compared to the control group by IHC and WB. Expressions of CTGF and TGF-β1were increased after the first 2-week immobilization and then decreased by ISH, and increased gradually by IHC compared to the control group.
Conclusions: The expression of mRNAand protein levels of collagen types I and III were not increased after immobilization, which indicated that accumulation of the two types of collagen was not the etiology of joint contracture. However, fibrogenetic factors of CTGF and TGF-β1 were increased. Another process, such as capsule and synovial adhesions or collagen cross-linking, may be possible causes of joint contracture.

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PROSTAGLANDIN D2 ENHANCES INTERLEUKIN -1BETA- INDUCED CYCLOOXYGENASE-2 EXPRESSION IN OSTEOARTHRITIC SYNOVIOCYTES

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Purpose: To investigate the effects of prostaglandin D2 (PGD2) on interleukin-1beta (IL-1beta)-induced cyclooxygenase (COX)-2 expression in human synoviocytes and the signalling pathways involved in these effects.
Methods: Synoviocytes were stimulated with IL-1 in the presence or absence of PGD2, and expression of COX-2 protein was evaluated by western-blotting. Messenger RNA (mRNA) expression was analyzed by real-time reverse transcription-polymerase chain reaction. The role of the PGD2 receptors D prostanoid receptor 1 (DP1) and chemoattractant receptor-like molecule expressed on Th2 cells (CRTH2) was evaluated using specific agonists.
Results: PGD2 increased in a dose-dependent manner IL-1-induced COX-2 protein and mRNA expression. DP1 and CRTH2 were expressed and functional in synoviocytes. The effect of PGD2 was mimicked by DK-PGD2 and Indomethacin, selective agonists of CRTH2, but not by BW245C, a selective agonist of DP1. Furthermore, treatment with an anti-CRTH2 antibody reversed the effect of PGD2, indicating that the stimulatory effect of PGD2 is mediated by CRTH2. Activation of CRTH2 is consistent with the activation of a receptor coupled to a phosphoinositide-specific phospholipase, suggesting that the effect of PGD2 is mediated by the CRTH2/PIP2/PKC.
Conclusions: PGD2 enhances IL-1-induced production of COX-2 by synoviocytes through the CRTH2/PIP2/PKC signalling pathway.

Matrix Biochemistry

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ELEVATED LEVELS OF COLLAGEN TYPE III IN ARTICULAR CARTILAGE OF OSTEOARTHRITIC JOINTS

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Purpose: To determine differences between well-defined OA and control human articular cartilage samples in type III collagen content.
Methods: Full-depth, intact surface cartilage from 5 OA (aged 60-80, 4 women) and 5 reference (aged 78-87, 5 women) femoral heads were obtained at total hip replacement surgery for osteoarthritis (OA) or femoral neck fracture.
1 mg/ml α-chymotrypsin plus inhibitors was added to each diced sample (incubated at 32°C overnight) to remove susceptible collagen components.
An aliquot of the supernatant was freeze-dried and used for SDS-PAGE and another aliquot was hydrolyzed in 6M HCl at 110°C to be assayed for hydroxyproline (μg/mg wet wt.) colorimetrically.
SDS-PAGE and Western blots were run using mAb 1C10, which recognizes a sequence-specific epitope in α1(II) CB9,7 and mAb 4G9, which recognizes a conformational epitope in the collagen III N-propeptide domain.
A competition ELISA was run on the same extracts using the 4G9 mAb to quantify the collagen III N-propeptide levels extracted from OA and reference cartilage samples.
Results: There was more extractable collagen in the OA than in the reference cartilage, 5% and 2%, respectively (p=0.02).
From OA and normals, most of the extracted collagen ran as intact α1(II) chains and large fragments on mAb 1C10 SDS-PAGE/Western (Fig. 2A).

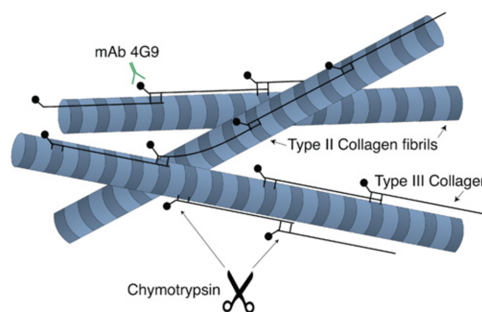

Figure 1. Model of Type III collagen polymerized on Type II collagen fibrils.

The major band stained in the SDS-PAGE/4G9 Western blot is the cleaved N-propeptide trimer of collagen III released from the matrix by α-chymotrypsin (Fig. 2B).
The ELISA results show a five-fold higher mean content of type III collagen in OA hip cartilage versus fracture hip controls (Fig. 2B).

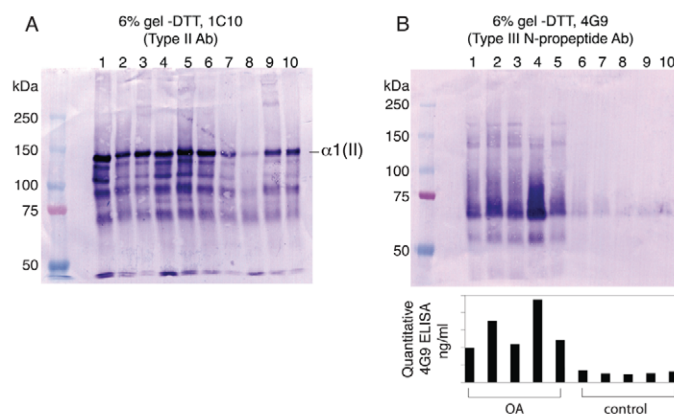

Figure 2. A. SDS-PAGE/Western blot of collagen type II in articular cartilage chymotrypsin extracts. B. SDS-PAGE/Western blot and an ELISA analysis of collagen type III N-propeptide domain released from articular cartilage by chymotrypsin.

Conclusions: Our study confirms previous results showing an increased pool of extractable collagen in OA. In the present study we additionally show that this extractable pool includes degradation products of type III collagen.
The results show that grossly normal looking cartilage from joints undergoing degenerative disease (OA) develops a significantly altered collagen phenotype as shown by the synthesized and deposited collagen III in the matrix of the OA compared with non-OA joints of similar age.
Previously it has been suggested that collagen III is made by the condrocytes in response to matrix damage similar to the wound-healing role of collagen III in type I collagen-based tissues.
The covalent addition of collagen III to the fibrillar matrix that occurs in both normal and OA human articular cartilage suggests an active remodeling process.
It remains to be established if increased amount of collagen III at sites of superficially intact, full thickness OA cartilage reflects pathological activity, an active repair mechanism or both.

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INFRAPATELLAR FAT PAD CAN INDUCE FIBROTIC PROCESSES IN CULTURED SYNOVIOCYTES

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Purpose: Stiffening of the joint is one of the features of knee osteoarthritis, which can be caused by fibrosis of the synovial tissue within the joint. In this study, we investigated whether the adipose tissue in the joint (infrapatellar fat pad) excretes cytokines and growth factors that could be involved in fibrosis.