INTRODUCTION:
The collagen network of human articular cartilage is built on a heteropolymorphic fibril template of type II (more than 90%), IX and XI collagens. Once laid down during development, there is little evidence that articular chondrocytes can recapitulate the overall collagen architecture if the mature tissue is damaged by injury or degeneration as suggested by the estimated turnover time of 400 years for human femoral head cartilage determined by the synthetic rate of hydroxyproline (1). However, components of the collagenous matrix may be remodeled more rapidly in response to mechanical and molecular signals without turnover of the bulk of the inter-territorial collagen. Indeed, experimental in vitro labeling studies suggest that new collagen molecules are formed (2). Previous studies in mature animal models of OA (3), indicated that type II collagen is the major product but there is evidence for other types, particularly type III collagen (5-7).

AIM:
In this study we sought differences between well-defined OA and control human articular cartilage samples in the content of type III collagen.

METHODS:
Five OA (aged 60-80, 4 women) and five reference (aged 78-87, 5 women) femoral heads were obtained at total hip replacement surgery for osteoarthritis (OA) or femoral neck fracture. Full-depth, intact surface cartilage from OA joints was removed from where it was possible to sample and in reference from superior weight-bearing part of femoral heads. 1 mg/ml α-Chymotrypsin plus inhibitors was added to each diced sample to remove susceptible collagen components. After incubation at 32°C overnight, supernatant was removed and aliquots of the supernatants were hydrolyzed in 6M HCl at 110°C overnight and then dried. Hydrolysates were re-dissolved in water, clarified with charcoal/AG-1 X8 anion exchange resin, and assayed for hydroxyproline (µg/mg wet wt.) colorimetrically. Remaining chymotrypsin extract was freeze-dried and aliquots used for SDS-PAGE and other analyses. SDS-PAGE and Western blots were run using mAb 1C10, which recognizes a sequence-specific epitope in α(II) CB9,7 and mAb 4G9, which recognizes a conformational epitope in the collagen III N-propeptide domain. A competition ELISA was also 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 both, most of the extracted type II collagen ran as intact α(II) chains and large fragments on mAb 1C10 SDS-PAGE/Western.

Fig. 1 shows the results of SDS-PAGE/4G9 Western blot and quantitative ELISA analyses of the chymotrypsin extracts of femoral head samples of articular cartilage from five reference (fracture) and five OA-hip cartilage determined by the synthetic rate of hydroxyproline (1). However, components of the collagenous matrix may be remodeled more rapidly in response to mechanical and molecular signals without turnover of the bulk of the inter-territorial collagen. Indeed, experimental in vitro labeling studies suggest that new collagen molecules are formed (2). Previous studies in mature animal models of OA (3), indicated that type II collagen is the major product but there is evidence for other types, particularly type III collagen (5-7).

DISCUSSION:
Our study confirms previous results showing an increased pool of extractable collagen in OA (4). 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 compared with cartilage from non-OA joints of similar age. Specifically, it appears that type III collagen is synthesized and deposited in the matrix by the articular chondrocytes. Previous studies suggest that this deposition is intimately associated with the collagen type II polymeric matrix. Recently it was shown that a pool of type III collagen is covalently cross-linked to the surface of collagen II fibrils in the form of pN-type III molecules (5). It was speculated that the type III collagen was made by chondrocytes in response to matrix damage similar to the wound-healing role of type III collagen in type I collagen-based tissues. In addition, it has been shown by transmission electron microscopy that type III collagen is co-localized with type II collagen in the same banded fibrils and retained its N-propeptide domain (6). In a study of OA cartilage, collagen III tended to be concentrated in the superficial and upper middle zones and to be synthesized by the chondrocytes (7).

CONCLUSIONS:
The covalent addition of type III collagen to the fibrillar matrix of articular cartilage in adult human joints suggests an active remodeling process. It occurs in both normal and OA joints, but the amount appears to be highly elevated in the latter at sites of superficially intact, full thickness cartilage. Whether this reflects pathological activity, an active repair mechanism or both will be important to establish.

REFERENCES:

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