DENATURED COLLAGEN IN OSTEOARTHRITIC BONE
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Introduction
The mechanical properties of connective tissue are influenced by the collagen quality. Osteoarthritic cartilage contains 4 times as much denatured collagen as intact cartilage(1). It is not known if this is due to mechanical damage or enzymatic degradation of the collagen.

Denatured collagen also affects the mechanical properties of bone(2). It has been demonstrated that osteoarthritic bone matrix is mechanically inferior to normal bone(3,4). While I is likely that reduced bone mineralization leads to the decline of mechanical properties in osteoarthritis, there is little known about the quality of the collagen matrix. Here, we test the hypothesis that the subchondral trabecular bone from human donors with mild to moderate cartilage damage has increased levels of denatured collagen. To begin to understand the possible mechanisms involved, we also performed experiments to test the ability of mechanical stimuli and enzymatic activity to induce collagen denaturation in mineralized bone samples.

Methods
This study consisted of two parts; testing of human cancellous bone from mildly arthritic donors and testing of bovine cortical bone.

Experiment 1 Human trabecular bone was harvested post mortem from the subchondral region of the proximal tibia. All donors had died suddenly from trauma or acute disease. Early arthritis was defined as macroscopically degenerated fibrillated cartilage and was confirmed histologically. The articular cartilage showed visual degeneration with slight fissures in the superficial zone of the medial condyle cartilage whereas the surface of the lateral condyle was intact. Mankin scores for normals were 2.0 or less and for tibiae with mild cartilage damage were between 3.0 and 7.0. Nine cancellous bone specimens were obtained using a water cooled trephine from either the medial and/or lateral compartment of 11 early-stage OA donors (average age: 75, range 63 – 87) and 13 normal controls (average age: 70, range 61–85). The sampling location was 5 mm distal to the subchondral bone plate on each side. All donor sample collection had been approved by an ethical review.

Experiment 2 Fresh bovine tibiae were obtained from the slaughter-house. Samples of diaphyseal compact bone were cut using a water cooled diamond saw. Specimens were then subjected to one of the following treatments: Experiment 2A i) untreated controls ii) fatigue loading in 3-point bending for 170000 cycles with a maximum stress of 80 MPa iii) incubation for one week in 2 mg/ml bacterial collagenase at 37°C. Experiment 2B i) untreated controls ii) crushing in a mortar and pestle to a size of approximately 2-3 mm iii) pulverization down to a fine powder using a Mikro-Dismembrator iii) submersion liquid nitrogen as a control for part of the pulverization procedure.

Denatured collagen was quantified using a previously described procedure(1). Briefly, samples were decalcified in EDTA before selectively digesting denatured collagen using alpha-chymotrypsin. The supernatant (containing the digested collagen) was then separated from the remaining insoluble matrix. Next the two pools were hydrolyzed and the amount of hydroxyproline in each sample was measured using either reverse phase high performance liquid chromatography (human specimens) or a colorimetric assay (bovine specimens). The ratio of hydroxyproline in the two pools could be used to quantify the amount of damaged collagen with respect to intact collagen in each sample.

Data were analysed using one way ANOVA and student t-tests.

Results
Experiment 1 In human subchondral trabecular bone there was a difference between groups (ANOVA p<0.04). There was a 22% increase in denatured collagen in the medial condyle of the osteoarthritic knees with respect to the controls p=0.04. There was a similar nonsignificant increase of 18% on the lateral side (p=0.11). There were no significant differences between the medial and lateral condyles of either group (Figure 1).

Experiment 2A Many fatigue specimens broke before 170000 cycles. After fatigue loading, there was no significant change in the amount of denatured collagen. While incubation in collagenase resulted in a measurable amount of denatured collagen in the incubation solution, there was no difference between the amount of denatured collagen in the remaining mineralized specimens and control specimens.

Experiment 2B Crushing bovine cortical bone in a mortar and pestle did not significantly increase the amount of denatured collagen.

However, pulverization doubled the denatured collagen content. Submerging the sample in liquid nitrogen did not effect the denatured collagen content (Figure 2).

Discussion
In the first part of this study we examined the amount of denatured collagen in subchondral trabecular bone of human donors. An increase in the denatured collagen content was noted in the presence of mild to moderate cartilage damage on the medial side. Surprisingly, this increase seemed to be present also on the lateral side where the cartilage was intact. We hypothesized increased denatured collagen could be due to i) microdamage ii) increased protease activity from the cartilage or iii) increased bone remodeling. Our in vitro experiments indicate that it is unlikely that the increase in denatured collagen is induced mechanically. Neither fatiguing nor crushing the samples had a measurable effect on the denatured collagen. Pulverization to a fine powder only doubled the amount of denatured collagen. Use of an aggressive bacterial collagenase did not increase the denatured collagen content. This is probably because of the multiple cleavage sites available to bacterial collagenase. However, this result indicates that osteoid is an unlikely source of denatured collagen (or unassembled collagen). Protease testing should be further pursued using human collagenases to better simulate in vivo conditions.

In conclusion, we observed an increase in the amount of denatured collagen in the subchondral trabecular bone of donors with mild cartilage damage. We were not able to induce collagen denaturation in vitro mechanically or enzymatically except for the extreme case of pulverization suggesting that other factors may be important.

Reference

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