# X-ray diffraction studies of the molecular substructure of human articular cartilage.

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## Introduction

Osteoarthritis is a prevalent disease that affects the cartilage in the joints of aging humans, having a serious impact on the quality of their lives. More than 40 million Americans are currently affected. A similarly large number of Americans have damaged joints because of work- or sport-related injuries. Articular cartilage provides a smooth gliding surface on diarthrodial joints that cushions the underlying bone. Only 5% of the tissue mass of a human adult joint consists of cellular components; everything else is extracellular matrix. Within this matrix, the main structural component is collagen type II fibers. The collagen fiber bundles have a diameter of about 60 nm and a length of several micrometers. They are grossly arranged in patterns similar to the pillars of gothic cathedrals, extending vertically from their base at the interface with the bone ("deep zone") and forming a cross vault underneath the superficial zone (which itself has horizontal layers of diagonally arranged fibers). It is believed that the vertical fibers contribute to guiding load vertically through the cartilage into the bone, whereas the horizontal fibers of the articular surface might guide lateral shear forces during joint motion into the adjacent connective tissue of the synovium. This gross orientation is supported by a network of randomly oriented short, very fine fibrils [1, 2]. This picture is largely hypothetical and is pieced together based on findings from animal tissue, from cartilage of different *in vivo* ages, and (for the most part) without any consideration of damage and repair status. Because of the wet, hydroscopic nature of cartilage, it is a very difficult tissue to study using electron microscopy.

Here, we show that small-angle diffraction can be used to study the distribution of collagen in cartilage from human knee and ankle joints and yield new insights into its function in normal tissue and in disease.

## Methods and Materials

Cartilage samples were harvested from the joints of organ donors or from arthroplastic surgery. The location of the cartilage on the joint was highly controlled; in most cases, cartilage from weight-bearing areas was used. In all cases, the cartilage was graded for the degree of grossly visible degeneration [3]. The tissue was fixed in paraformaldehyde but not further processed. For these studies, we used the Bio-CAT small-angle camera with doubly focused undulator beam with a 1–3 m camera and the 1024 x 1024 CCD detector. Exposure times were of order 1 s. The beam dimensions at the sample were about 250 x 800 microns (vertical x horizontal). The sample was scanned through the beam with a 2 micron resolution X-Z stage using a video-equipped stereo microscope to locate the specimen. The cartilage specimens were scanned vertically from the superficial to the deep zone in 0.1 mm increments.

## Results

In our preliminary studies of these tissues, we concentrated on the gross collagen fiber orientation. Nonosteoarthritic and nondegenerated ankle cartilage has an average height of about 1.5 mm. This cartilage contained a surface layer that was about 300 micron thick with collagen fiber orientations of about 15 degrees from the horizontal ("horizontal fibers"). This layer was followed by a 400 micron layer of midzone cartilage with no preferred fiber orientation, and a deep layer of additional 400 microns with fibers at an angle of 85–95 degrees ("vertical fibers"). (See Figure 1.) Between these layers, there were about 200 micron thick transition layers where the orientation gradually rotated. In normal knee cartilage (about 3 mm in height), the thickness of the upper and the bottom layer remained almost identical to the ankle. However, the midzone layer expanded to almost 2 mm.

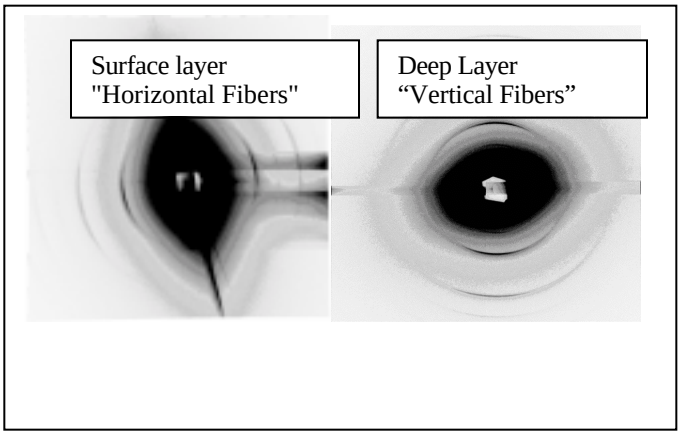


Figure 1: Surface layer collagen fibers ("horizontal fibers") and deep layer collagen fibers ("vertical fibers") from ankle cartilage.

In moderately damaged ankle cartilage, the pattern changed dramatically. First, if the damage caused a loss of the superficial zone, the preferential horizontal orientation of the fibers at the surface remained. In other words, the fiber orientation of the former midzone was reorganized to form a new layer of horizontally aligned collagen fibers. Additionally, in severely damaged cartilage, we observed a loss of vertical organization in the deeper layers, possibly indicating the proteolytic destruction of collagen by matrix metalloproteases or an additional reorientation of the fibers

to compensate for the loss of the midzone structure, now transformed into a "surface" structure.

## Discussion

These results have not been seen before and are providing us with unique insights into the distribution and remodeling of collagen fibers in this tissue. We expect to obtain additional structural information on the association of collagens with proteoglycans from future experiments. These data will deepen our knowledge on the structural basis of the biomechanical behavior of cartilage. Our preliminary data also suggest that the current dogma of an "inert" collagen fiber network cannot be maintained since we observe major fiber reorientation during damage and degeneration. In other words, we can monitor and quantify disease- and treatment-related changes on the level of molecular macro-organization in the extracellular matrix. This technique will also allow us to determine the efficacy of experimental medical interventions such as drug treatments and tissue transplantation, with respect to the molecular functionality of the repair or replacement tissue. A more detailed report on these results is in preparation for publication.

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