INTRODUCTION: Information on the structure of normal and diseased cartilage has been challenging to obtain because of the complex composition and high degree of hydration of this tissue that have made it a difficult subject for electron microscopic studies (1). Within the cartilage matrix, the main structural component is collagen type II fibers, arranged in bundles having a diameter of about 60 nm and a length of several micrometers. The gross orientation of the collagen network is largely hypothetical, being pieced together from histological findings from animal tissue, from cartilage of different in vivo ages, and, for the most part, without any consideration of damage and repair status. One method that can be used to non-invasively investigate the structural arrangements of collagen fibers in this system is small-angle X-ray diffraction. Since earlier X-ray studies were performed with tissue from various species from birds to human and without controlling age and health status, the findings are difficult to interpret. Another difficulty in the earlier work is the danger of drying artifacts due to the long exposure times (minutes to hours) required to obtain X-ray patterns. Here we show that by using intense, highly collimated X-rays at the Advanced Photon Source, we have been able to obtain (in a few seconds) spatially-resolved information of collagen fibers in the matrix of normal and osteoarthritic cartilage from the ankle and knee joints.

METHODS: Human normal, degenerated and osteoarthritic cartilage was harvested within 24 hours of surgical removal or death of the donor. Full thickness samples of ~10 by 10 mm were excised and fixed in paraformaldehyde or kept in saline at 4°C for up to 72 hours. Cartilage was graded for the degree of grossly visible degeneration (2). Sample morphology was documented using a Zeiss Stemi 2000 stereomicroscope equipped with a Cohu model 2122 video camera. Small-angle x-ray diffraction experiments were performed using the BioCAT undulator based beamline at the Advanced Photon Source, Argonne National Labs, Argonne IL. The undulator beam was doubly focused with a 1-5 m specimen to detector distance and an x-ray beam energy set to 12 keV (1.03 Å wavelength). Exposure times were of order 0.2-3 seconds. The sample was scanned through the beam with a 2 micrometer resolution vertical and horizontal stage using a video-equipped stereomicroscope to locate the specimen. X-ray patterns were collected using a CCD-based x-ray detector (1024x1024 pixels, 60 mmx60 mm active area). Spacings on detector images were digitally measured using the program FIT2D (4) or with NIH Image. The tissue samples were then processed for microscopy, including picrosirius red staining. The 8 µ sections were observed by polarizing microscopy for comparison with collagen fiber orientation as obtained through small-angle x-ray diffraction. The relative sign of birefringence was determined by turning the slide in two opposite directions. For ordinary light microscopy, the polarizer was removed from the light path.

RESULTS: The figure shows the averaged orientation angle as a function of depth from the surface in normal ankle cartilage. The collagen fibers are parallel to the surface in a very narrow layer ~200 µ thick just under the surface (superficial zone). There is a more isotropic distribution just below that (mid-zone) for ~400 µ and then the fibers become approximately perpendicular (85-95°) to the cartilage bone interface (deep zone) for ~500 µ. Between these layers, there were ~200 µ thick transition layers where the orientation gradually rotated. Data from the upper and bottom layers of the knee cartilage was almost identical to that of the ankle. However, the midzone expanded to almost 0.2 mm in thickness. In moderately degenerated ankle cartilage, the pattern changed dramatically from the normal. When the degeneration caused a loss of the superficial zone, there was a preferential horizontal orientation of the fibers at the surface. Hence, the fiber orientation of the former mid-zone was, perhaps, collapsed thus resulting in a new superficial region of horizontally aligned collagen fibers. In addition, in severely degenerated cartilage, there was a loss of vertical organization in the deeper layers, possibly indicating a proteolytic destruction and reorganization of collagen or a collapse of the fiber network throughout. Polarizing microscopy reflected the same pattern observed in the x-ray diffraction of the same specimens. In particular, the normal collagen fiber orientation was highly altered resulting in areas of alternating orientation or areas of no preferred orientation in osteoarthritic cartilage.

DISCUSSION: The high flux density and relatively high energy of the x-ray beam have allowed us to quickly and easily collect spatially-resolved collagen fiber patterns in relatively thick samples of normal and osteoarthritic articular cartilage. The high throughput (0.2-2 seconds exposure time per image) allowed us to examine a reasonably large number of clinically relevant samples. Our findings from normal, undamaged cartilage are largely in accord with the postulated vault distribution based on electron microscopic data. An important finding is the observation from osteoarthritic cartilage that there can be extensive collagen fiber network collapse or reorganization resulting in a much thicker layer of horizontal fibers only switching to vertical fibers very close to the bone. These structural rearrangements may be expected to significantly alter the mechanical properties of this tissue. Another unique finding is that the transition layer is thicker in knee cartilage in comparison to ankle. These data will extend our knowledge on the structural basis of the biomechanical behavior of normal and damaged cartilage, a necessary prerequisite for any form of “tissue engineering”. Further studies must investigate the mechanism of collagen fiber re-orientation observed in degenerated cartilage to decipher whether or not it is the result of network collapse or active re-arrangement. Thus, we have shown that one can monitor and quantify disease- or treatment-related changes on the level of molecular macro-organization in the extracellular matrix.


**Illinois Institute of Technology, Chicago, IL 60616.