

Correlation of Collagen Organization with Polarization Sensitive Imaging of in Vitro Cartilage: Implications for Osteoarthritis

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ABSTRACT. *Objective.* Optical coherence tomography (OCT), a new method of high resolution imaging, has shown feasibility for assessing articular cartilage to identify early changes in osteoarthritis (OA) and monitor therapy. OCT is analogous to ultrasound, measuring the intensity of backreflected infrared light rather than sound. The resolution of this technology is up to 25 times higher than existing methods. We investigated the correlation between changes observed by OCT and the degree of collagen organization in OA cartilage.

Methods. Polarization sensitive OCT (PSOCT) imaging was used to assess changes in cartilage collagen organization *in vitro*.

Results. The presence (or absence) of PSOCT changes correlated with collagen organization (or disorganization) on histology as assessed by picrosirius polarization microscopy (no significant difference). In multiple cases, cartilage was abnormal by both PSOCT and polarization microscopy, but was grossly normal by routine staining, showing cartilage thickness > 2 mm and no fibrillations.

Conclusion. This *in vitro* study suggests PSOCT changes in cartilage are due to the state of collagen organization. The combination of high resolution structural imaging and birefringence detection make OCT a potentially powerful technology for early assessment of OA. (J Rheumatol 2001;28:1311–8)

Key Indexing Terms:

OSTEOARTHRITIS ARTICULAR CARTILAGE IMAGING TOMOGRAPHY
OPTICAL COHERENCE COLLAGEN MAGNETIC RESONANCE IMAGING

Recent advances have suggested that the progression of articular cartilage damage in osteoarthritis (OA) may be modified via interventions such as tissue transplantation, chondrocyte transfer, biological matrices, or pharmaceuticals^{1–4}. This has led to a need for better methods to visualize early changes in cartilage and monitor the progression of

these changes. While conventional radiography is a useful and inexpensive method for determining extensive disease, its sensitivity is limited for assessing early disease and monitoring therapy. Magnetic resonance imaging (MRI) has shown considerable promise for assessing articular cartilage. However, its limited resolution (over 100 μm) makes it difficult to reproducibly measure early articular changes⁵. Further, it is questionable whether it would be cost effective to broadly apply MRI to this large patient population who typically have multijoint involvement, even if early stages of the disease could be detected. Arthroscopy is impractical due to its invasive nature and inability to assess cartilage substantially below the articular surface. High frequency ultrasound has recently been examined for intraarticular use, but due to its limited resolution (roughly 80–90 μm at 40 MHz) and large arthroscopy size, its application is not likely to represent a substantial advance^{6,7}.

A recently developed technology, optical coherence tomography (OCT), has shown considerable promise as a method of high resolution (4–20 μm), minimally invasive intraarticular imaging^{8,9}. OCT is analogous to ultrasound, measuring the back reflection of infrared light rather than sound waves. It was originally developed to image the transparent tissue of the eye^{8,10}. Recently, OCT has been developed for high resolution imaging in nontransparent

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tissue^{11,12}. In addition to its high resolution, OCT is attractive for intraarticular imaging for several reasons. First, OCT is high speed, with current data acquisition rates up to 16 frames per second^{13,14}. Second, OCT endoscopes are small and consist of just simple fiber optics. This would mean the procedure could be performed in a physician's office or at bedside, similar to the introduction of curette biopsy in gynecology¹⁵. And unlike ultrasound endoscopes, they contain no transducer within their frame, making them relatively inexpensive¹³. Finally, the OCT system is compact and portable, about the size of an ultrasound machine.

Recently, imaging of *in vitro* and *in vivo* osteoarthritic joints has been performed with OCT and several significant observations were noted⁹. First, the thickness of cartilage has been delineated to below 10 μm . Second, fibrosis, small fibrillations, and abnormalities in the subchondral plate could be identified. Finally, it was noted that normal cartilage yielded polarization sensitive imaging, while osteoarthritic cartilage did not. Polarization sensitive OCT (PSOCT) imaging means that the backreflection signal from within the tissue changed with changes in the polarization state of the incident light. This does not occur with most tissue, where normalized backreflection does not change with the polarization state of the incident light. It is known that organized collagen is birefringent by light microscopy and the hypothesis is that this is the mechanism behind PSOCT of normal cartilage. Since collagen becomes disorganized in the progression of OA, the loss of polarization sensitivity likely results from breakdown of the collagen architecture. Since collagen disorganization precedes gross changes such as fibrillations and cartilage thinning, a technology capable of monitoring changes in collagen organization could be a powerful modality for the early detection and monitoring of OA. We examined the relationship between the degree of polarization sensitivity during OCT imaging of *in vitro* cartilage and changes seen in collagen examined by picrosirius polarization sensitive microscopy. The hypothesis is that these variations in imaging will be correlated with disorganization of collagen. Ultimately, the longterm hypothesis is that the greatest potential of PSOCT lies in early detection of osteoarthritic changes.

MATERIALS AND METHODS

OCT measures the intensity of backreflected infrared light as a function of depth in a manner analogous to ultrasound^{8,11}. The time for light to return to the detector, or echo delay time, is used to measure distances. To produce cross sectional tomographic images, the light beam is scanned across the tissue. The result is 2D or 3D data sets of the optical reflectance properties of the tissue. A schematic of the OCT system is shown in Figure 1.

Because of the high speed of light propagation, direct measurement of the echo delay time cannot be made electronically. To measure the delay time (and optical path length) OCT uses a technique known as low coherence interferometry. The light is coupled into an optical fiber. The light is evenly split by an optical beam splitter. In this embodiment, half the light is directed toward a moving reference mirror, half at the sample. The distance to the moving mirror is precisely controlled. Light reflected from

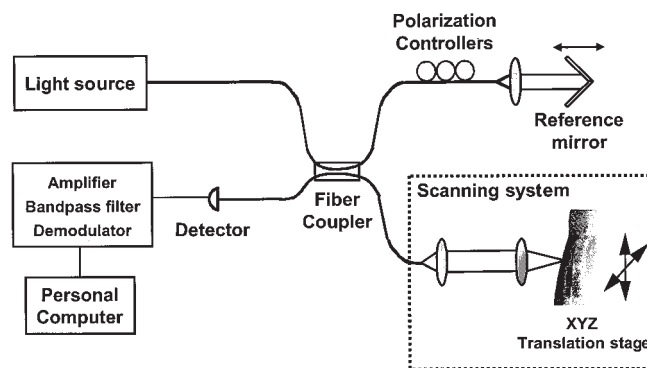


Figure 1. Optical coherence tomography system consists of a fiber optic based Michelson interferometer using a low coherence light source.

structures within the tissue is combined with light reflected from the mirror. Interference occurs only if the path light travels in both arms (optical path length) is almost identical. OCT measures the intensity of interference and uses this to represent the intensity of back reflection. The signal from the sample will only be measured if it has traveled the same distance as light in the reference arm has traveled (to within the coherence length). By moving the mirror, the distance light travels in the reference arm is changed, which allows information from different depths within tissue to be obtained.

A 1300 nm superluminescent diode was used, which had a bandwidth of 45 nm, corresponding to an axial resolution of 18 μm . A transversal resolution of 30 μm was achieved using a 30 mm lens that focused the beam onto the sample. The sensitivity of all measurements was 110 dB. The polarization state of light in the reference arm was changed with polarization controllers. Imaging was obtained at the same site with light in different polarization states. Two dimensional imaging was achieved by scanning the specimen with a movable stage (X, Y, and Z), which has steps of 1 μm . Digital imaging data was analyzed with IPLab Software (Signal Analytic, Vienna, VA, USA). Sampling is roughly at 10 $\mu\text{m}/\text{pixel}$.

Twenty-four articular surfaces, obtained either post-limb amputation or post-joint resection, were imaged *in vitro*. Over 70% were either the talus or metatarsal joints. Because the images generated by OCT represent very high resolutions, correlation with histology can be difficult, particularly since the source is infrared and invisible to the eye. Tissue registration remains critical for comparing OCT images of fine structure to histology. For this reason, a visible light guiding beam has been installed and imaging plains are marked with microapplications of dye. The planes are marked first and then the OCT beam is scanned from the point of maximal width in one dye spot to the maximal width on the other dye spot. Typically, the tissue is cut 1 mm from the section of interest (parallel to the imaging plane), then the sample is sectioned with a microtome until the area of interest is reached (marked area). This avoids damage to the site imaged by the initial cut, in addition to allowing more precise tissue registration. The slice used for correspondence is one where the width of the dye is maximum. With this technique, we have achieved cellular level correlations¹⁶.

Post-imaging, the samples were fixed in formalin and then slowly decalcified with an EDTA based protocol. After routine tissue processing, the samples were stained with sirius red F3B or picrosirius staining. Picrosirius staining has been shown to be superior to conventional polarization microscopy for the identification of collagen and its organization within joints^{17,18}.

Since this is the first study examining polarization sensitivity, the grading scale was based predominately on reasoning, using our understanding of the physics and limited experience. Undoubtedly, as the phenomena are better understood, modifications of this grading scale will be required. This issue is addressed in the discussion. OCT images were scored by a blinded investigator on a scale of 0, 1, or 2. Polarization

changes are defined by movement of polarization bands with changes in the polarization state of incident light. For samples graded 2, the movement of entire polarization bands exceeds 50% of the width of the image when the polarization was scanned. For samples scored 1, polarization bands, over at least two-thirds the length of the image, moved within 10–50% the width of the image. A zero corresponded to no noticeable change in the image (< 10%) with changes in the polarization of incident light or no polarization bands present.

Organized collagen appears as a bright region via picrosirius staining. Its color is more greenish for thin fibers (0.8 μm) and yellowish-orange for thick fibers (1.6–2.4 μm)¹⁹. In normal samples, when the surface is at maximum, there is a slight decrease in intensity in the deepest layer due to the orientation of the collagen. While scoring systems exist for picrosirius staining in the literature, they do not adequately address the phenomena being presumed to be measured with OCT. This issue is addressed in the discussion. Samples stained with picrosirius red were graded by a blinded investigator, who assigned a score of one of 3 numbers. All measurements were performed with an Olympus microscope (BX40) with a polarization filter (UPOT) and polarization analyzer (U-AN360). A grade of 2 represents highly polarized and means that a contiguous region of bright intensity was present within 100 μm of the surface, was > 50% the width of the cartilage, and contained no significant gaps. A grade of 1 corresponded to a region of bright intensity of between 25 and 50% of the total cartilage thickness with gaps \leq 20% of the length or the width of the cartilage. A zero did not meet either of these criteria.

The goal of these 2 grading systems is to grossly correlate OCT images with picrosirius staining at the levels of normal (collagen not disorganized, 2), mild–moderate (significant collagen disorganization, 1), and severe (almost completely disorganized, 0). When quantitative systems are developed, more precise correlations will be made.

All values are means plus the standard error. To establish differences between groups, a 2 tailed t test using paired data sets was used. The level of significance for each type of analysis was set at 0.05. Statistical analysis was blinded to treatment.

RESULTS

The score of each image is listed in the figure legends. Figure 2 shows imaging of cartilage that is grossly normal by both nonpolarized microscopy and picrosirius staining. A section of cartilage from the distal tibia (shown by arrows) is seen abutting an adjacent region of supportive tissues. The 3 images on the left are taken with light at different incident polarization states. It can be seen that as the polarization is changed, the bands within the image change, which is the definition of polarization sensitive imaging. Of note, these bands are not Benninghof arcades per se, but represent a rotation of the polarization states of the backreflected light. The cartilage is grossly normal by nonpolarized microscopy (panel d) and highly positive for polarization sensitive staining by picrosirius staining (e). In Figure 3, a series of OCT images of the articular surface of the talus show polarization sensitive imaging is present. However, it is not as uniform as in Figure 2. The corresponding nonpolarized microscopy slide shows grossly normal cartilage (d). While the sample shows strong birefringence seen in the picrosirius red staining (e), it is not as homogeneous as in Figure 2, possibly explaining the nonuniformity of the OCT imaging.

In Figure 4, no significant polarization changes are seen in the OCT images of a femoral condyle. The nonpolarized microscopy slide shows grossly normal cartilage that is

thick with no obvious fibrillations. Picrosirius staining shows only minimal birefringence, with virtually none on the surface. This is consistent with the hypothesis that polarization sensitive changes are due to changes in collagen orientation. Similarly, in Figure 5, there is no significant polarization sensitivity in the OCT images and minimal birefringence is seen in the picrosirius stained section. Again, while the nonpolarized microscopy slide does have abnormal staining, it is thick with no significant fibrillations, so that changes would likely be difficult to detect with structural imaging.

Table 1 lists all samples scored for both OCT and picrosirius. For OCT, there were 14 scores of 0, 7 scores of 1, and 3 scores of 2. For picrosirius, there were 15 scores of 0, 6 scores of 1, and 3 scores of 2.

The mean score for all the OCT images was 0.5 ± 0.2 . The scores for all the picrosirius stained samples read by a blinded investigator were 0.5 ± 0.2 mean. There was only one sample that differed in score between the OCT images and the blinded investigator, so that there was no significant difference between these groups.

DISCUSSION

Collagen in healthy cartilage is highly organized^{20,21}. While the exact arrangement varies from joint to joint and person

Table 1. Samples scored for picrosirius. and OCT staining.

	Histology	OCT
1	1	1
2	1	1
3	0	0
4	0	0
5	0	0
6	0	0
7	0	0
8	0	0
9	1	1
10	2	2
11	0	0
12	0	0
13	1	1
14	0	0
15	0	0
16	0	0
17	2	2
18	0	0
19	1	1
20	0	0
21	1	1
22	0	1
23	2	2
24	0	0

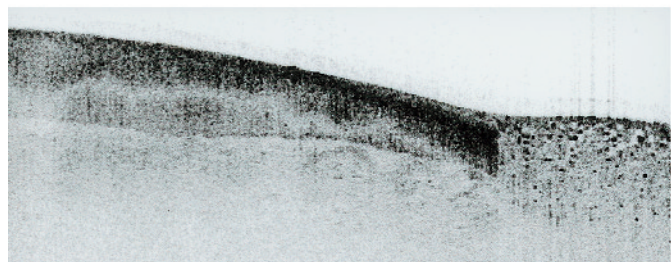
Grading: 2 = normal (collagen not disorganized); 1 = mild to moderate (significant collagen disorganization); 0 = severe (almost completely disorganized).



A

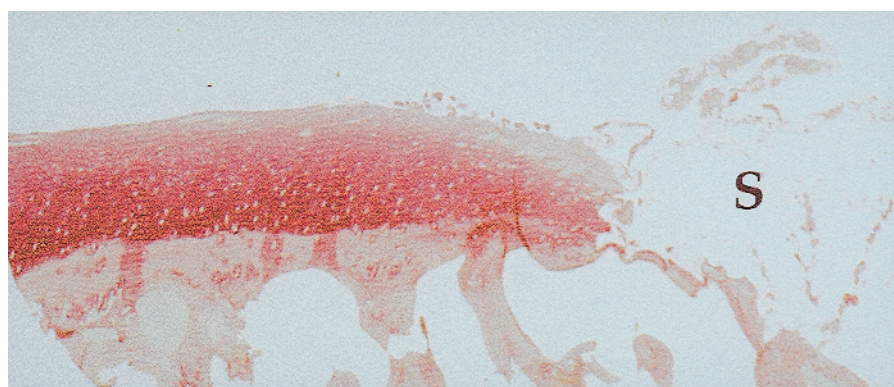


B

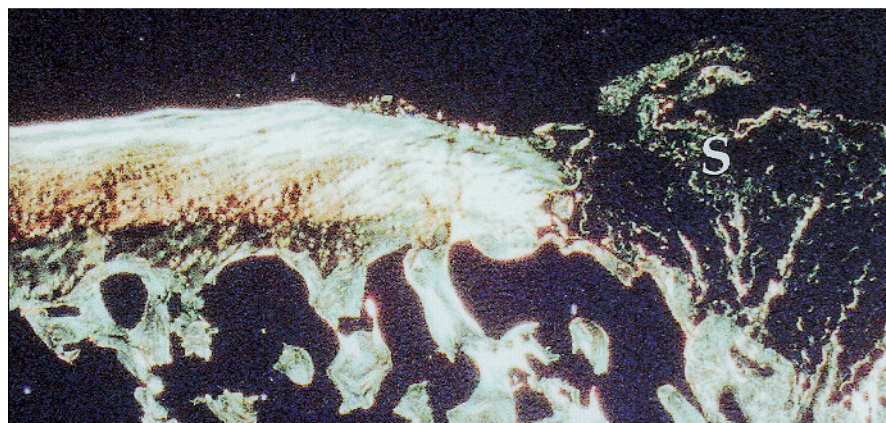


C

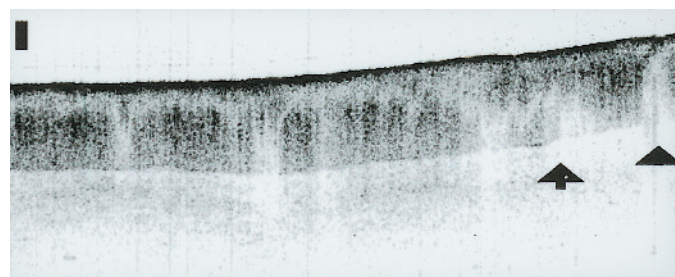
Figure 2. OCT images recorded with different polarization states at the same site of the distal tibia (indicated by arrows) with adjacent supportive tissue (S) and corresponding histology (panels D, E). The supportive tissue, which contains fat cells, has been slightly disrupted in the histology. Change of the white polarization band in the cartilage with change of polarization indicates high polarization sensitivity that correlates well with the grossly normal appearance of the cartilage by nonpolarization histology (D) and highly positive for polarization sensitive staining (E). Bar = 500 μ m. Both the histology and OCT image were scored 2 because they both met the criteria.



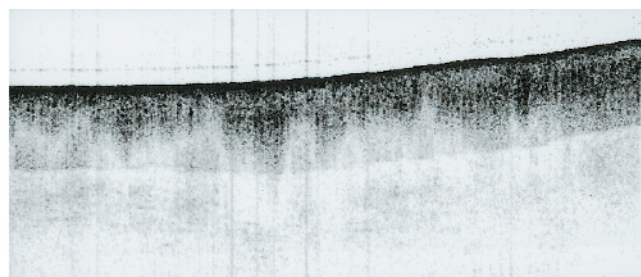
D



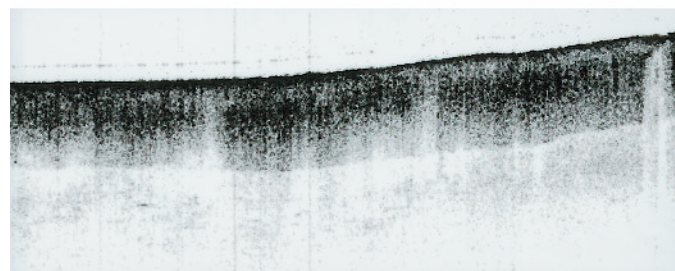
E



A

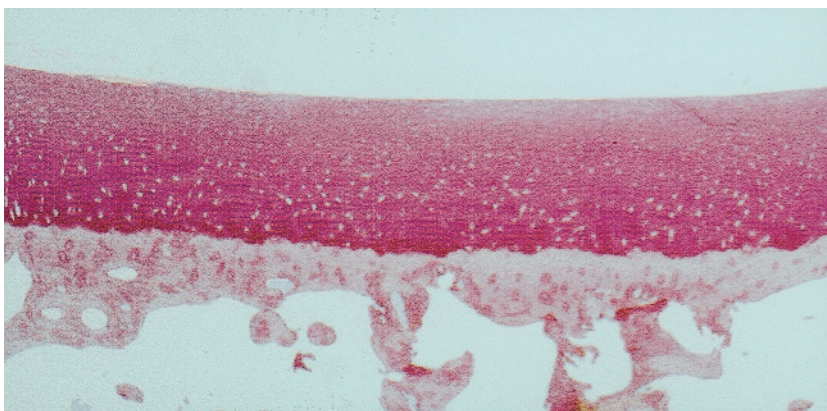


B

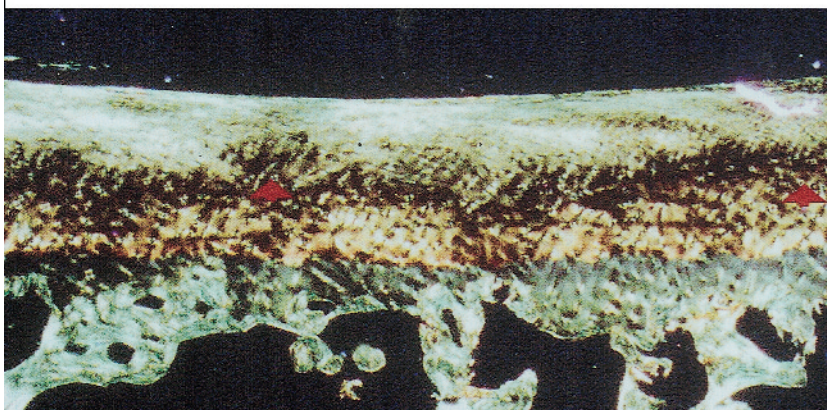


C

Figure 3. OCT images (panels A–C) with 3 different polarization states and the corresponding histology (D, E) of talus cartilage, show polarization sensitive OCT imaging, which is slightly irregular compared with Figure 2. The corresponding nonpolarization section (D) indicates grossly normal cartilage. While the picrosirius stained section is strongly birefringent, it is less homogeneous than Figure 2, likely leading to the irregularity in the OCT image. Bar = 500 μ m. This image received OCT score = 1 because the right side did not move 50% of the image (A, black arrows). The histology scored 1 because there were significant gaps and sections that did not extend over 50% of the cartilage (E, red arrows).



D



E

to person, Benninghoff's model has generally been widely accepted^{20,21}. In this model, there are 3 regions within the cartilage, a superficial region parallel to the surface, a medial region that is transitional, and a deep region that is perpendicular to the surface. Changes in collagen orientation, although not necessarily content, are among the earliest changes in OA. In animal models, decreased birefringence by histology precedes fibrillations and can even be noted after chronic long distance running^{22,23}.

We correlated polarization sensitive changes in OCT images of cartilage with changes in cartilage collagen organization. The study is limited by the difficulty in finding samples with a grade of 2 in this patient population. A strong correlation was noted between birefringence in OCT images and blinded investigator's assessment of picrosirius stained sections. The only sample with a difference had a single section that showed high birefringence by histology, and it is likely OCT measurement was predominately in this region. Samples of greatest interest were those with cartilage thickness > 2 mm and no gross fibrillations, yet no birefringence was detected by OCT or picrosirius staining. Two examples are Figures 4 and 5. These samples are consistent with the hypothesis that loss of birefringence in OCT images may be an early marker of OA. The presence or absence of bands was not due to geography, as both diseased and normal samples were obtained from the talus and metatarsal joints. This is not an *in vitro* artifact, as the presence of birefringence has been shown *in vivo* recently in humans²⁶.

The authors needed a gross method for defining normal, mild to moderately diseased, and severely diseased of both the histology and OCT images. Since this is the first study examining polarization sensitivity, the OCT grading scale was based predominately on reasoning dependent on our understanding of the physics and limited experience. For this reason, only a gross 3 point scale was used. "Normal" was defined in terms of the movement of polarization bands over 50% of the width of the image when the polarization was scanned. The 50% value was used rather than 33% because it was assumed that there would be a large variation in "normal" and it was likely that some early disease could still exceed 33%. Future studies will need to refine this system, particular as quantitative systems are developed and larger numbers of patients are examined. However, it should be pointed out that the grading system, designed prior to interpretation, did match the picrosirius in all but one sample in this blinded study. While it can be argued that this is due to the design of the histologic grading system, the reasoning behind this scale, described below, is not likely to result in large errors, particularly since it is just a gross 3 point scale.

Previous investigators have scored picrosirius samples based on quantitative measurements of the total intensity measured with a CCD camera. We examined a similar system based on a Matlab script but elected not to use it,

once again based predominately on reasoning dependent on our understanding of the physics and limited experience. This system was inadequate for several reasons. First, from experience, severely diseased cartilage with fibrous bands can have a relatively high score based on this scale, but by any scoring system would be abnormal. Second, regional disruptions, particularly near the surface, tend to occur with early OA, but can still have total intensities within the normal range. "Normal," in this study representing a contiguous region of bright intensity present within 100 μm of the surface, was greater than 50% the width of the cartilage, and contained no significant gaps. The 50% value was chosen rather than a higher number, for example 90%, because generally the deeper regions of collagen will show up darker at baseline because of their different orientation. Therefore normal cartilage will almost never be over 90%. Due to the gross nature of the OCT grading system, this system was also limited to a 3 point scale.

Future work will focus on quantitating birefringence in OCT images, developing a smaller OCT arthroscope, increasing the data acquisition rates, and confirming data with *in vivo* studies. This study used a 3 number scoring system for OCT images. There is a current quantitative technique for PSOCT that uses 2 detectors^{24,25}. The major disadvantage of this technique, in addition to the fact that it is not imaging based, is that significant power losses occur in the system due to beam splitters and filters that would make it difficult to perform imaging at high data acquisition rates. Future single detector systems will quantitatively measure the rate of change of polarization by rotating the polarization of the incident light at a constant rate. This will allow rapid and more precise measurement.

In addition, future work will develop small OCT arthroscopes. The current OCT endoscope, which is 2.9 French, was designed for imaging within the vascular system and the gastrointestinal tract⁸. Since the core optical fiber is only 124 μm in diameter, arthroscopes can likely be designed capable of fitting through the lumen of a 21 gauge needle. The fastest OCT imaging is currently up to 14 frames per second^{8,14}. Future modifications are needed to get the system at video rate. Finally, *in vivo* clinical studies are needed to confirm *in vitro* results and to assess its utility for monitoring the course of osteoarthritic changes.

This study describes a correlation between polarization sensitive changes by optical coherence tomography and the degree of collagen organization in cartilage. Since this is a relatively small study with a gross qualitative scoring system, studies on a larger scale with quantitative OCT technology are needed to determine degree and subtleties of the correlation. However, this study does suggest a potential role for OCT in monitoring changes in early OA independent of gross structural changes such as cartilage width and fibrillations.

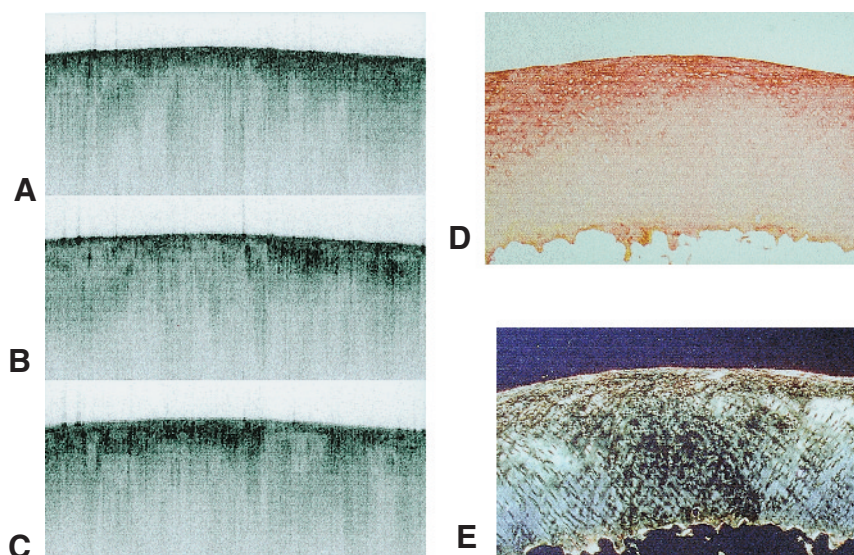


Figure 4. OCT images (panels A–C) and corresponding histology (D, E) of a femoral condyle. No significant polarization changes were detected in the OCT images, while the H&E stained section (D) revealed grossly normal, thick cartilage with no obvious fibrillations. Picrosirius staining (E) revealed only minimal birefringence. Bar = 500 μ m. This image received a zero OCT score because there was less than a 10% change in the polarization bands. The histology scored zero because it did not have sufficient intensity to score 1 (i.e., bright intensity between 25 and 50% of the total cartilage thickness with gaps no greater than 20% the length or the width of the cartilage). The bands (or dropout areas) within the image could represent isolated areas of organized collagen within the image, but this is speculation.

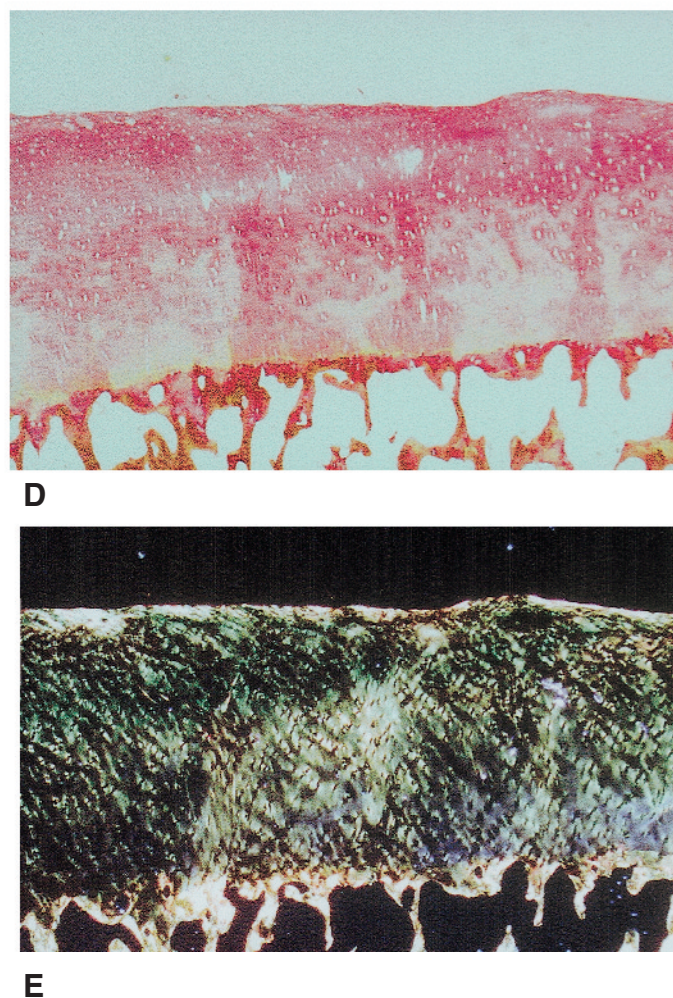
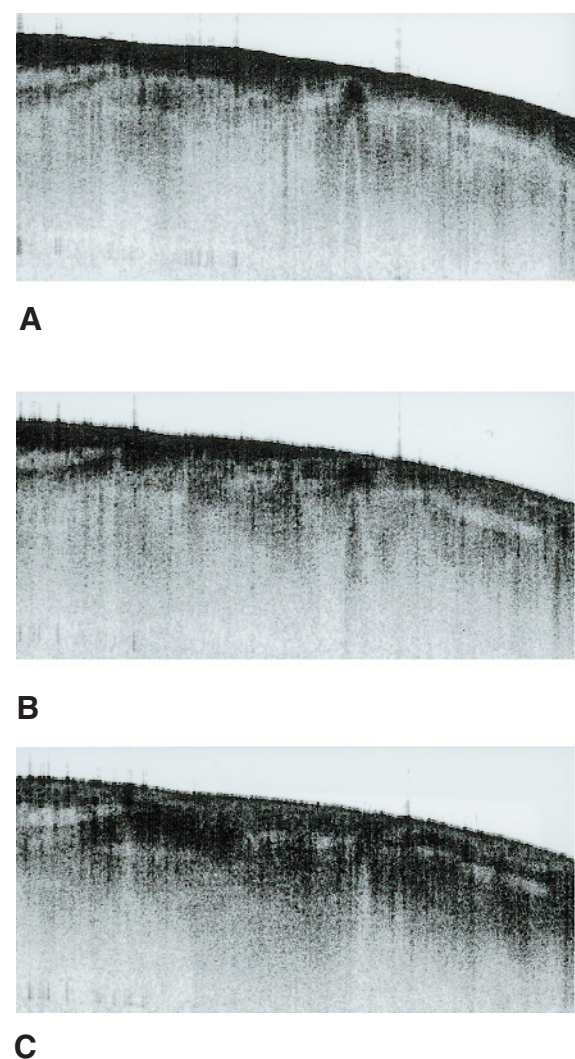


Figure 5. OCT images (panels A–C) and corresponding histology (D, E) of a femoral condyle. No significant polarization sensitivity is seen in the OCT images or in the picrosirius stained section (E). Nonpolarization stained histology section reveals a thick, normal appearance with no significant fibrillations. Bar = 500 μ m. This image also scored a zero, for the same reasons as in Figure 4. The bands (or dropout areas) within the image could represent isolated areas of organized collagen within the image, but this is speculation.

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