Clinical diagnosis of potentially treatable early articular cartilage degeneration using optical coherence tomography

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Abstract. A series of bench to operating room studies was conducted to determine whether it is feasible to use optical coherence tomography (OCT) clinically to diagnose potentially reversible early cartilage degeneration. A human cadaver study was performed to confirm the reproducibility of OCT imaging and grading based on identification of changes to cartilage OCT form birefringence using a polarized OCT system approved for clinical use. Segregation of grossly normal appearing human articular cartilage into two groups based on the presence or absence of OCT form birefringence showed that cartilage without OCT form birefringence had reduced ability to increase proteoglycan synthetic activity in response to the anabolic growth factor IGF-1. The bench data further show that IGF-1 insensitivity in cartilage without OCT form birefringence was reversible. To show clinical feasibility, OCT was then used arthroscopically in 19 human subjects. Clinical results confirmed that differences to OCT form birefringence observed in *ex vivo* study were detectable during arthroscopic surgery. More prevalent loss of cartilage OCT form birefringence was observed in cartilage of human subjects in groups more likely to have cartilage degeneration. This series of integrated bench to bedside studies demonstrates translational feasibility to use OCT for clinical studies on whether human cartilage degeneration can be diagnosed early enough for intervention that may delay or prevent the onset of osteoarthritis.
[DOI: 10.1117/1.2789674]

Keywords: coherent optical systems; biomedical optics; clinical study; tomography; articular cartilage; osteoarthritis.

Paper 07044SSRR received Feb. 6, 2007; revised manuscript received Jun. 27, 2007; accepted for publication Jun. 27, 2007; published online Oct. 19, 2007.

1 Introduction

Osteoarthritis is reaching epidemic proportions as our population ages.[1] While osteoarthritis is of multifactorial etiology and eventually involves the entire joint, the central pathologic feature has traditionally been attributed to progressive loss of articular cartilage.[2] Efforts to prevent disabling disease are hampered by difficulty in identifying cartilage degeneration before the onset of irreversible changes. The earliest signs of cartilage degeneration include potentially reversible metabolic changes that begin prior to breakdown of the articular surface.[3] Cartilage with these earliest signs of degeneration cannot be currently identified clinically with magnetic resonance imaging (MRI) or arthroscopy. Nondestructive methods that can diagnose human cartilage degeneration at these early and potentially reversible stages may lead to new strategies to modify the progression of cartilage degenerative processes.

Optical coherence tomography (OCT) can be used to image articular cartilage with structural clarity comparable to low-power histology.[4–7] OCT is a cross-sectional imaging technology analogous to ultrasound except that OCT generates an echograph of infrared light at resolutions comparable to histology.[8] Fiberoptic OCT can also be incorporated into arthroscopic surgery to assess animal and human articular cartilage.[4,9]

In human tissue and cadaver studies, arthroscopic OCT was substantially more sensitive than conventional arthroscopic surface imaging in identifying microstructural breakdown of the articular surface.[4] When compared to histopathology, visual inspection of the surface underestimated the degree of damage, while OCT detected fine surface fibrillations. OCT image data can also be used to assess subsurface microstructure as denoted by changes to the multilaminar banding patterns seen in normal articular cartilage.[4,10] In an arthroscopic porcine study, the dark bands forming the layered appearance could be altered by changing the polarization angle of the OCT, indicating that they represent a phenomenon known as OCT form birefringence.[9]

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1083-3668/2007/12(5)/051703/6/$25.00 © 2007 SPIE

Observations that grossly intact appearing cartilage from osteoarthritic knees frequently show disrupted OCT banding patterns suggest that changes to OCT form birefringence may indicate early cartilage degeneration.[9–11] Chondrocyte insensitivity to the anabolic effects of growth factors has been implicated in the pathogenesis of osteoarthritis.[3]

Several recent studies show that this early pathological change can be potentially reversed.[12,13] Thus, we believe that OCT may be translated from bench to operating room for identification of potentially reversible early pathological changes found in articular cartilage degeneration. If this is true, then OCT may prove to be a useful tool for basic and clinical studies on the treatment and reversal of early cartilage degenerative processes.

The central hypothesis of this paper is that it is feasible to use OCT clinically to detect changes to OCT form birefringence suggestive of potentially reversible early cartilage degeneration. To test this hypothesis, we conducted an integrated series of bench to operating room studies to first evaluate the reproducibility of OCT scanning and grading and to determine whether metabolic alterations in human articular cartilage with loss of OCT form birefringence are reversible, based on the ability to increase proteoglycan production in response to IGF-1. To show feasibility for arthroscopic use, we then conducted a clinical study to determine whether the same changes to articular cartilage OCT form birefringence observed in *ex vivo* studies can be observed during *in situ* clinical imaging of human subjects undergoing arthroscopic surgery.

2 Materials and Methods

These integrated bench to operating room studies were performed using a clinical OCT system (Imalux, Cleveland, Ohio) to determine the presence or absence of OCT form birefringence in fresh human articular cartilage, in human cadaver knees, and during arthroscopic surgery in 19 human subjects. Human cadaver knees were used to determine whether detection of cartilage OCT form birefringence is reproducible using clinical OCT, fresh human articular cartilage was used to determine whether loss of OCT form birefringence correlates to potentially reversible metabolic insensitivity to IGF-1, and OCT imaging of human subjects during arthroscopic knee surgery was performed to determine whether loss of OCT form birefringence of the medial femoral condyle is more prevalent in individuals with degenerative medial meniscus tears, which are a clinical sign of early joint degeneration,[14–16] compared to individuals with other types of intra-articular pathology.

2.1 *OCT Imaging*

All OCT imaging was performed using a 1300-nm fiberoptic polarized OCT system (Imalux) approved by the Food and Drug Administration (FDA) for clinical use.[17,18] This clinical OCT system has a lateral resolution of 25 μm and an axial resolution of 15 μm. Image acquisition is 1.5 s for a 200 $\times$200 pixel image. The cartilage was scanned by rotating the OCT probe through four different radial orientations approximately 45 deg apart.

2.2 *Reproducibility of OCT Image Acquisition*

Two surgeons independently performed OCT scanning of 33 designated study areas to the medial and lateral femoral condyles and to the trochlea of three human cadaver knees from elderly donors (average age 75) with no known history of knee problems and no joint space narrowing on unloaded AP radiographs. The joint surfaces of the knees were examined, and the cartilage to the medial and lateral femoral condyles of all three knees appeared intact to gross examination. While two knees had surface fibrillations to the trochlear groove, there were no areas of complete cartilage loss. Study areas (Fig. 1) were designated by scoring the cartilage using an 8-mm scoring device. OCT scans were obtained *in situ* from the designated study areas to the intact distal femur. Each surgeon obtained 4 scans per study area, for a total of 264 OCT scans. Neither surgeon was present for the OCT imaging performed by the other surgeon. OCT scans were randomized for independent blinded grading by two independent observers. The 4 OCT scans for each study area were grouped, yielding 66 OCT quartets. Each quartet was stripped of all identifying information, randomized, and independently graded by each grader according to the criteria of whether or not OCT form birefringence was present (Fig. 1). The presence of OCT form birefringence was defined as observation of a distinct banding pattern creating a multilaminar appearance in at least one of the four orientations [Fig. 2(a)]. Specimens that did not have distinct dark bands in any of the four orientations were considered to be without detectable OCT form birefringence [Fig. 2(b)].

Agreement between OCT grades was tested using unweighted kappa.[4,19]

2.3 *Metabolic Studies*

Grossly normal appearing articular cartilage specimens from the femoral condyles of osteoarthritic knees were obtained at the time of total knee replacement and used in accordance to an exempt protocol approved by the university's Institutional Review Board (IRB) and Committee for Research in the Dead (CORID). The tissue was OCT scanned within 4 h of harvest and separated into two groups based on whether or not OCT form birefringence was detected.

The cartilage was sharply separated from the subchondral bone and cut into 2 to 5-mm pieces. The tissues were weighed and aliquoted into 24 well plates for sextuplicate analysis of each specimen. Cartilage pieces were cultured for 24 h in 10% FBS. Inhibition of nitric oxide was performed through administration of 1 mM NG-monomethyl-L-arginine (L-NMA), an inhibitor of nitric oxide synthase to cartilage with and without OCT form birefringence during this initial 24-h incubation. Nitric oxide release into the media was quantified using the Greiss reaction.[13] The specimens were then washed and cultured in serum-free media for 48 h with and without 50 ng/ml of IGF-1. Following growth factor stimulation, proteoglycan synthetic activity was assessed by measuring 35S-sulfate incorporation as previously described.[12,20] Comparison was made between results from cartilage with and without OCT form birefringence using the unpaired t-test with $\alpha = 0.05$.

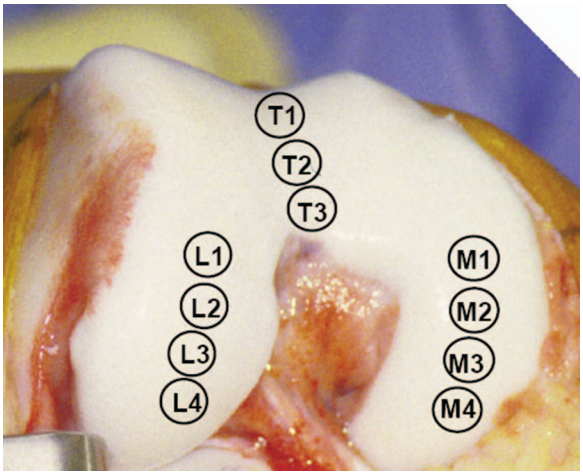

Fig. 1 Human knee joint with circles showing the designated study areas to the medial femoral condyle, the lateral femoral condyle, and the trochlea.

2.4 *Clinical Study*

Human subjects scheduled for arthroscopic knee surgery were recruited and clinical data used according to protocols approved by the university's Institutional Review Board. Individuals with greater than 50% narrowing of the tibiofemoral joint spaces in weight-bearing anteroposterior radiographs were excluded from the study. During arthroscopic surgery, the same study areas to the medial femoral condyle M1 through M4 (Fig. 1) used in the cadaver study were examined using conventional arthroscopic surface imaging and probe palpation. OCT imaging was performed arthroscopically of study areas with intact articular surfaces using the same rotational scanning technique as for the cadaver study. Twelve individuals were treated for degenerative medial meniscus tears identified by preoperative history and MRI scan and seven individuals underwent arthroscopic surgery for other intra-articular pathologies. Degenerative meniscus tears are described as complex, horizontal and flap tear patterns that constitute tearing of the central substance of the meniscus occurring after insignificant or low-energy trauma.[15,16] The other seven subjects underwent arthroscopic surgery for two lateral meniscus tears, one post-traumatic vertical tear to the medial meniscus, two anterior cruciate ligament tears, one diagnostic arthroscopy, and one chondral defect of the lateral femoral condyle. There were 10 males and 9 females ranging in age from 24 to 80 years. While individuals with degenerative meniscal tears had an average age of 56 compared to an average age of 48 in those with other diagnoses, the difference in age was not significant ($p=0.11$). Clinical OCT images underwent blinded grading for the presence or absence of OCT form birefringence at least four weeks after the images were obtained. Statistical analysis was by Chi square testing with significance set at $\alpha<0.05$.

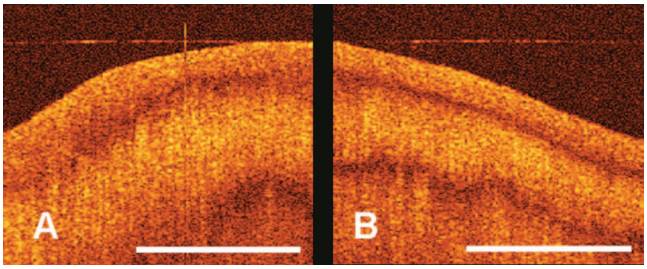

Fig. 4 Arthroscopically acquired clinical OCT scans (medial femoral condyle, M1) of a 28-year-old male undergoing arthroscopic repair of a traumatic medial meniscus tear showing clear OCT form birefringence. Note the slight change to the multilaminar banding pattern in these two scans of the same study area acquired at different orientations. Scale bar=1 mm.

3 Results

3.1 *Reproducibility of OCT Scanning and Grading*

OCT image quartets of each study area (Fig. 1) independently acquired by two surgeons received the same blinded grade from both graders for 30/33 (91%) of the study areas (unweighted $\kappa=0.85$).

3.2 *Metabolism*

While basal proteoglycan synthetic levels were similar, specimens with grossly intact articular surfaces that had no observable OCT form birefringence exhibited insensitivity to the anabolic effects of IGF-1. In specimens retaining cartilage OCT form birefringence, IGF-1 increased proteoglycan synthesis by 95% ($p<0.05$). This anabolic response was not

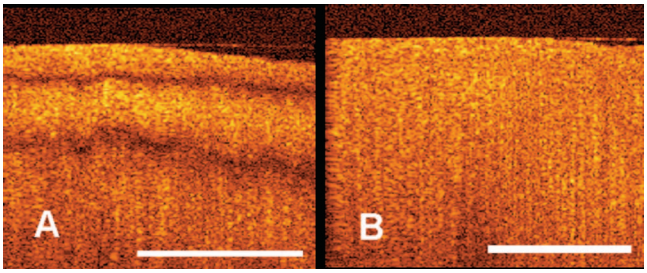

Fig. 2 Cartilage OCT form birefringence. (a) OCT image of cartilage with OCT form birefringence where distinct dark bands create a multilayered appearance. (b) OCT image of cartilage without OCT form birefringence. In cartilage graded to be without OCT form birefringence, there were no recognizable banding patterns in any of the four scan orientations. Scale bar=1 mm.

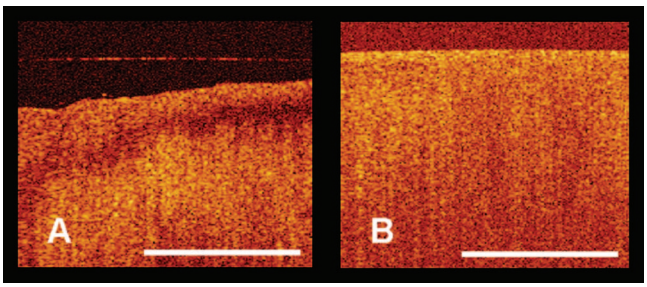

Fig. 5 (a) Arthroscopically acquired clinical OCT scan (medial femoral condyle, M2) of a 49-year-old male after anterior cruciate ligament tear showing the presence of OCT form birefringence, and (b) of a 47-year-old male with a degenerative medial meniscus tear without detectable OCT form birefringence (medial femoral condyle, M2). Scale bar=1 mm.

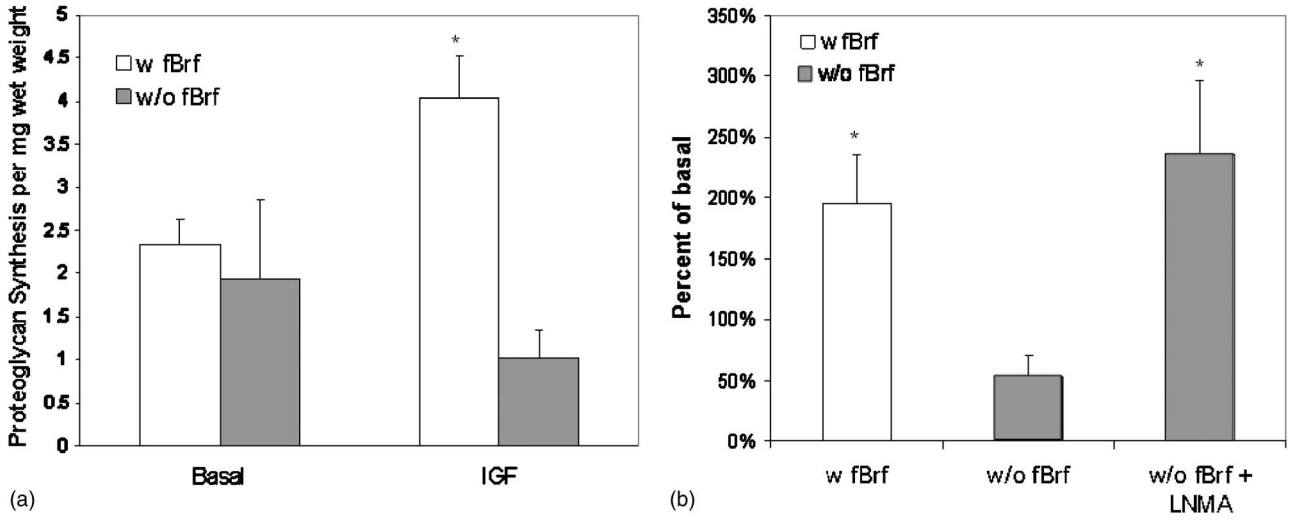

Fig. 3 Reversal of cartilage insensitivity to IGF-1. (a) Specimens with normal cartilage OCT form birefringence (white) increased proteoglycan synthesis in response to IGF-1. While basal proteoglycan synthetic activity was similar for cartilage with and without OCT form birefringence, cartilage without normal cartilage OCT form birefringence (gray) was insensitive to the anabolic effects of IGF-1. (b) Inhibition of nitric oxide synthase using L-NMA restored the anabolic response of cartilage without OCT form birefringence (gray) to IGF-1.

observed in specimens without OCT form birefringence [Fig. 3(a)]. Nitric oxide (NO) has been implicated in chondrocyte insensitivity to IGF-1, and measured NO levels were 50% higher in cartilage without OCT form birefringence ($p < 0.05$). Incubation of specimens with L-NMA lowered the NO released into the medium from 51 pM/mg tissue to 22 pM/mg tissue ($p < 0.05$). In L-NMA–treated tissue that had lost OCT form birefringence, IGF-1 stimulated PG synthesis by 137% ($p < 0.05$), demonstrating restoration of IGF-1 responsiveness [Fig. 3(b)].

3.3 *Clinical Study*

OCT images to the medial femoral condyle were successfully obtained arthroscopically through the standard anteromedial arthroscopic portal using a fiberoptic OCT probe (Figs. 4 and 5). Clinical imaging of articular cartilage was consistently obtained to a depth of 1.5 mm. In clinical study, the distinct banding patterns previously observed in *ex vivo* study of some human articular cartilage specimens were readily seen in multiple scan orientations of the same study area in multiple human subjects (Fig. 4). The same alterations to OCT form birefringence consisting of inability to see birefringence in any of four scan orientations were also observed clinically. Following blinded grading of OCT images, the percentage of study areas to the medial femoral condyles without OCT form birefringence was found to be more prevalent in individuals with degenerative patterns of medial meniscus tears occurring in 97% of study areas compared to 26% of study areas in individuals with traumatic medial meniscus tears, lateral meniscus tears, or chondral defects not affecting the medial femoral condyle ($p < 0.001$) (Fig. 5). Loss of OCT form birefringence was also more prevalent in individuals 50 years of age or older occurring in 89% of study areas than in individuals younger than age 50, where this occurred in 11% of study areas ($p < 0.03$).

4 Discussion

The results of these integrated bench to operating room studies support the hypotheses that clinical OCT can be used to reproducibly detect changes to cartilage birefringence indicative of potentially reversible early cartilage degeneration and that clinical OCT can be successfully used arthroscopically to identify differences in OCT birefringence patterns. In this study, cartilage without OCT form birefringence demonstrated reduced ability to increase proteoglycan synthetic activity in response to IGF-1 and elevated nitric oxide levels. The bench data further show that the observed IGF-1 insensitivity could be reversed by blocking nitric oxide synthase. The clinical finding that loss of OCT form birefringence was more prevalent in the medial femoral condyles of individuals with degenerative medial meniscus tears, a clinical marker for early joint degeneration, compared to individuals with other types of intra-articular pathologies supports further clinical study to complement the laboratory findings that loss of OCT form birefringence is a potential marker for early cartilage degeneration.

While detection of cartilage OCT form birefringence has been reported previously using both polarized OCT and polarization-sensitive OCT,[6,9–11,21] the reports have been variable, and the structural basis for the observed birefringence is not well understood. It is known that the polarization state of the OCT light source will alter this pattern, and that [PSOCT] may offer improved detection of changes to cartilage birefringence.[9–11] For clinical translation, imaging systems approved by the Food and Drug Administration (FDA) for clinical use frequently do not incorporate technologies available in the laboratory setting.[22–25] In focusing on the goal of clinical translation, the available FDA-approved equipment was that of a polarized OCT system. Working within the equipment limitations, we sought to determine whether the presence or absence of OCT form birefringence as detected by the clinically available OCT system was reproducible.

To determine reproducibility of OCT scanning and grading, two surgeons independently scanned 33 designated areas, *in situ*, in the normal anatomical positions of human cadaver knees. The results of blinded grading indicated that the clinical OCT system reproducibly detected cartilage OCT form birefringence when the same area of cartilage was imaged by different individuals at different times. Demonstration of reproducibility of *in situ* OCT imaging and grading of human articular cartilage is important to supporting potential clinical use of OCT in the evaluation of articular cartilage.

Chondrocyte insensitivity to anabolic growth factors has been implicated in the pathogenesis of osteoarthritis and can occur prior to breakdown of the articular surface.[3,12,13,27] In focusing the use of OCT on cartilage subsurface changes that are not detectable by conventional arthroscopic surface imaging, OCT was used in this study to segregate cartilage with intact articular surfaces into cartilage with and without form birefringence observable using the same fiberoptic polarized OCT system approved for clinical use. Consistent with these studies, we found reduced ability to increase proteoglycan synthesis following administration of IGF-1 in human cartilage without OCT form birefringence. Our data further show that detectable changes to OCT form birefringence may be predictive of reversible chondrocyte insensitivity to the anabolic effects of IGF-1.

Microstructural changes associated with potentially reversible metabolic perturbations cannot currently be diagnosed clinically except by histopathology, which is not practical for early diagnosis because it requires removal of the cartilage being examined.[28] Although OCT can provide nondestructive optical images of human articular cartilage similar to histology,[4] potential limitations of OCT for clinical assessment of articular cartilage include the imaging depth of approximately 1 to 1.5 mm, permitting cross-sectional evaluation of only the superficial portion of human articular cartilage. This means that OCT is not well suited for evaluating cartilage degeneration extending through the entire tissue. These partial- and full-thickness lesions can be visualized by conventional arthroscopy, and also potentially by MRI.[26] However, as shown in the laboratory and cadaver studies, OCT may fill an imaging gap for early diagnosis of cartilage degeneration because OCT was able to show differences in cartilage form birefringence within the first 1 mm of depth in human articular cartilage with grossly intact articular surfaces.

The clinical study using OCT during arthroscopic surgery in 19 human subjects confirmed that OCT can be used clinically to identify the same changes to cartilage OCT form birefringence that in the laboratory study were shown to be predictive of potentially reversible early cartilage metabolic incompetence. We also showed, in cadaver study, that OCT imaging and grading were reproducible between different surgeons and different graders. This integrated series of bench to bedside studies demonstrates the potential and feasibility of using OCT in both basic and clinical imaging of human articular cartilage to assist in the development and implementation of new strategies for early diagnosis and treatment of human articular cartilage degeneration.

Acknowledgments

The authors thank Nicole Papas, Lesa Lewis Werkmeister, and John Karpie, MD, for their assistance. Funded by NIH Grant No. R01 AR046995 (CRC), the Arthritis Foundation (CRC), and the University of Pittsburgh Department of Orthopedic Surgery.

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