S. D. Martin · N. A. Patel · S. B. Adams
M. J. Roberts · S. Plummer · D. L. Stamper
M. E. Brezinski · J. G. Fujimoto

New technology for assessing microstructural components of tendons and ligaments

Accepted: 8 January 2003 / Published online: 5 February 2003
© Springer-Verlag 2003

Abstract This study investigated the ability of optical coherence tomography (OCT), a recently developed technology with micron-scale resolution, to assess the microstructure of tendons and ligaments. In vitro structural- and polarization-sensitive OCT was performed on human ACL, Achilles tendon, and biceps tendon (obtained postmortem). Histology was performed on all imaged samples and compared to the corresponding OCT data. OCT images correlated well with histology. Most importantly, through polarization-sensitive OCT, the collagen in normal tissue was easily distinguished from the surrounding, supportive tissue due to the birefringent properties of organized collagen. Since the integrity of collagen is an important indicator of structural stability and pathologic state, the ability of OCT to assess collagen could be a powerful diagnostic tool in assessing tendon and ligament properties.

Résumé Cette étude a examiné la capacité de la tomographie de cohésion optique (OCT), une technologie récemment développée avec une résolution de l'échelle du micron, à étudier la microstructure des tendons et ligaments. L'étude in vitro a été exécutée sur le ligament croi-

sé antérieur humain, le tendon d'Achille et le tendon du biceps (obtenu post mortem). L'histologie a été exécutée sur tous les échantillons et comparée aux données OCT correspondantes. Les images OCT ont eu une bonne corrélation avec l'histologie. Plus important, à travers l'image OCT en polarisation, le collagène du tissu normal a été distingué facilement des tissus avoisinants grâce à ses propriétés biréfringentes. Puisque l'intégrité du collagène est un indicateur important de la stabilité structurelle ou d'un état pathologique, la capacité de l'OCT à apprécier le collagène pourrait être un outil diagnostique puissant dans l'étude des propriétés des tendons et ligaments.

Introduction

Abnormalities of tendons and ligaments can lead to significant morbidity, including but not limited to pain, rupture and tear [1, 2, 9, 10, 12, 17]. The vulnerability of tendons and ligaments to develop pathology has been attributed to relative avascularity, mechanical trauma, and collagen degradation. Achilles and ACL rupture have been linked to underlying prior histopathological abnormalities of collagen, such that the collagen fibers become nonparallel, exhibit decreased striation, and vary irregularly in dimension [1, 2, 12, 17]. In addition, the pathophysiology of many disorders remains unknown, such as the chronic pain in the patella that often occurs following ACL replacement [9, 10]. While biopsy is a viable diagnostic option, it may be hazardous, create additional pathologies, or nonyielding if the correct location is not sampled. MRI, ultrasound, and arthroscopy all have major roles in assessing tendons and ligaments, but these technologies are not optimal at assessing pathologic changes at a histological level. The ability to analyze these structures on a micron scale could be a powerful tool for experimental analysis, in order to gain better insight into the pathophysiology of these disorders, and as a potential diagnostic modality.

S. D. Martin · N. A. Patel · S. B. Adams · M. J. Roberts
S. Plummer · D. L. Stamper · M. E. Brezinski
Department of Orthopedic Surgery,
Brigham and Women's Hospital and Harvard Medical School,
Boston, MA, 02115 USA

S. Plummer
Department of Biology, King's College,
Wilkes-Barre, PA, 18711 USA

J. G. Fujimoto
Research Laboratory of Electronics and Department
of Electrical Engineering and Computer Science,
Massachusetts Institute of Technology,
Cambridge, MA, 02139 USA

M. E. Brezinski (✉)
Orthopedics Research, Brigham and Women's Hospital,
75 Francis Street, Boston, MA, 02139 USA
e-mail: mebrezin@mit.edu
Tel.: +1-617-2332802, Fax: +1-617-2539611

Optical coherence tomography (OCT) has been previously introduced for the evaluation of articular cartilage [5, 6]. OCT is analogous to ultrasound, measuring the backreflection of infrared light rather than sound [4, 7]. OCT has several advantages as a method for assessing the musculoskeletal system. First, OCT has a resolution up to 25× higher than any technology in clinical medicine. While most OCT systems have an operating resolution between 10–20 μm , resolutions as high as 4 μm have been achieved in nontransparent tissue [3]. Second, OCT is fiber based, so that probes and arthroscopes can be very inexpensive and can be engineered to have very small diameters. The current smallest OCT probe is 400 μm in diameter [11]. Third, OCT performs imaging in real time, allowing large amounts of data to be collected [15]. Fourth, OCT can be obtained over distances of 2–3 mm, which is larger than a traditional excisional biopsy. Finally, OCT can be combined with a range of spectroscopic techniques including polarization sensitivity (PSOCT) [5].

PSOCT is extremely effective in identifying organized structures, such as healthy collagen [5]. When a highly organized structure is present, the properties of the image change with alterations in the polarization state of incident light due to a property of the tissue known as birefringence (i.e., the tissue has different indices of refraction depending upon the polarization of the light, causing the polarization state of light to change with propagation in the tissue). Most tissue is minimally polarization sensitive, since it does not contain the appropriate organizational structure. However, tendons and ligaments are composed of highly organized collagen. Thus, polarization sensitivity can be a sensitive indicator of early collagen disorganization and degradation.

In this study, OCT was performed on tendons and ligaments. Both structural imaging and polarization spectroscopy was performed. Our objective was to investigate the ability of OCT as an imaging modality to provide information regarding the microstructure of tendons and ligaments with future implications to the clinical setting.

Methods

The theory behind OCT has been previously described [4]. Briefly, ultra-short infrared pulses or low coherent light is directed onto the sample to be imaged. The time delay for the light to be reflected back, or the echo delay time, is used to measure distances of internal structures in the tissue. The intensity of backreflection is plotted as a function of the echo delay time or depth of the reflection from within the tissue. The beam is then scanned across the sample to produce two- and three-dimensional data sets. The data is displayed as a false color or gray-scale image, which represents a cross section through the tissue.

Due to the high speeds associated with the propagation of light, the echo delay time cannot be measured electronically. Therefore, a technique known as low coherence interferometry is required, where echoes of light from the sample are correlated with light that has propagated a known reference path delay in a reference arm. In low-coherence interferometry, light from the light source is split into a reference and sample arm, as shown in Fig. 1. Light reflects from the mirror in the reference arm and

Fig. 1 Schematic of the optical coherence tomography (OCT) system

from within the sample. When light from both arms travels the same optical distance, interference occurs as the two light beams recombine. The interference signal is detected electronically, and the amplitude of the interference is a measure of the reflected light intensity. By scanning the path length of the reference arm, light echoes from different depths in the tissue can be measured.

The axial resolution of OCT is dependent on the coherence length of the light source. In this study, the axial resolution was 16 μm , as measured from the point-spread function of a single reflection from a mirror. The lateral resolution was 30 μm , as determined by the focused spot size of the optical beam on the specimen. Imaging was performed at ~1300 nm wavelengths in the near infrared. The signal-to-noise ratio was 109 dB.

Achilles tendons (three), biceps tendons (two), and anterior cruciate ligaments (two) were obtained from randomized patients within 24 h either postmortem or postsurgical resection. Each sample was imaged at sequential points throughout its length for a maximum of 12 locations. The tendon samples were absent of gross pathology. One Achilles sample was obtained in the ruptured state (postsurgical resection). The samples were stored in 0.9% saline with 0.1% sodium azide at 3°C. Samples were placed on a translation stage, which permitted the incident OCT beam to be scanned in a transverse direction to generate a two dimensional cross-sectional image. For imaging, the uncut tendons and ligament surfaces were exposed to the OCT beam. The position of the invisible infrared light beam on the sample was monitored with a collinear visible light-guiding beam. At each location, images were obtained at three different states of light polarization. The peripheral areas of the imaged specimens were marked with microinjections of dye to permit comparison of the OCT images with histologic images. Samples were fixed in 10% neutral-buffered formalin for 12 h. Tissue sections were stained with hematoxylin/eosin (H/E), Masson's trichrome blue, or picrosirius red (for determination of collagen alignment) [14]. Stained histological sections were visually compared with OCT images to allow correlation.

Approval for the use of human tissue specimens was obtained from the Institutional Review Boards of the Massachusetts Institute of Technology and Brigham and Women's Hospital.

Results

The OCT images (Fig. 2a, b) are of a biceps tendon imaged longitudinally at one site with two different polarization states of the incident light. OCT effectively demarcates the layers of the tendon (C, S). The banding

Fig. 2 Longitudinal optical coherence tomography (OCT) images (a, b) and picrosirius-red staining (c) of the biceps tendon. OCT images are of the same site with two different polarization states. The banding pattern represents organized collagen (C) that is surrounded by supportive tissue (S)

Fig. 3 Cross section of a biceps tendon imaged at two different polarization states (a, b) and corresponding picrosirius-red staining (c). The collagen (C) is easily distinguished from the surrounding supportive tissue (S)

pattern (C) results from the birefringent properties of the organized collagen and shifts with respect to other structures in the image as the polarization state changes. The supportive tissue (S) around the collagen shows minimal polarization sensitivity. Fig. 2c is the same sample stained with picrosirius red and viewed with a polarizing filter. The bright region is birefringent due to the presence of organized collagen.

In Fig. 3, the biceps tendon is imaged in cross section. A pattern of high intensity backreflection is localized to the area containing collagen bundles but not the supporting tissue.

Similar images were obtained when the Achilles tendon was imaged (not shown). Areas that possessed a high degree of birefringence with picrosirius staining also demonstrated a pronounced banding pattern in the

Fig. 4 Optical coherence tomography (OCT) images (a–c) of unruptured ACL with corresponding picrosirius-red staining (d). The

banding pattern present in the OCT images corresponds with the area of organized collagen seen with histology

Fig. 5 Optical coherence tomography (OCT) images (a–c) of ruptured ACL with corresponding picrosirius-red staining (d). The collagen appears to be not as highly organized based on the ab-

sence of a banding pattern in the OCT images as well as an attenuation in the birefringence of the picrosirius-red staining

OCT image. As with the ligament, this banding pattern shifted with different polarization states of the incident light.

In Fig. 4, OCT images of an unruptured section of an ACL are shown with the corresponding histology. Again, a moving banding pattern is present in the OCT images. In contrast, Fig. 5 demonstrates the ruptured end of the ACL. It can be seen that polarization sensitivity (banding pattern) is completely lost in the OCT images, which corresponds with a dramatic reduction in the birefringence of the picrosirius stained image.

Discussion

From this work, OCT demonstrates promise as a method for assessing the microstructural organization of tendons and ligaments. The highly organized nature of the collagen and resulting birefringence of this tissue makes polarization-sensitive OCT a potentially powerful tool for identifying pathology. OCT can identify organized collagen within a tissue without excision, as occurs with scanning electron microscopy (SEM) and histology, and with higher resolution than MRI or ultrasound.

The banding pattern seen in the OCT images is consistent with the presence of organized collagen, as determined by birefringence in picrosirius-red-stained samples. This dye binds to collagen fibers in a parallel fashion and can be used to determine both the arrangement and thickness of collagen fibers present in a tissue [8, 14]. In healthy tissue, the OCT banding pattern changes with adjustments in the polarization state of the incident light or the polarization of light in the reference arm (Fig. 2a, b). These changes are most likely due to the polarization of the incident light changing as it propagates through the tissue. This produces a banding pattern when the backscattered light has a polarization state that is either aligned or orthogonal to the polarization state of the reference beam. The spacing between the bands ultimately may be used to further quantify the tissue's birefringence, with narrower spacing likely corresponding to higher tissue birefringence. The supportive tissue around the collagen shows minimal polarization sensitivity (both in OCT images and the corresponding histology).

It is important to note that each band is not necessarily equivalent to an individualized group of collagen but rather a reflection of the organized arrangement of collagen. The bands are representative of the extent of collagen organization and for each individual sample retain the same thickness with the same spacing between. Changing the polarization state of light results in a shifting of the banding pattern. While this study did not address disease progression, it is reasonable to hypothesize that serial OCT analysis of progressive collagen disorganization would depict changes in band thickness and interspacing until all organization is lost and the banding pattern is no longer evident (Fig. 5 a–c).

The scope of this work was imaging normal tissue in vitro with the inclusion of a limited number of pathologi-

cal samples. Future studies will focus on a more extensive correlation of images with progressive states of pathology, in vivo imaging, increasing the acquisition rate, and combination of OCT with other imaging techniques. Future studies also will utilize various spectroscopic techniques to improve the diagnostic information gained from OCT. In particular, OCT elastography will be used, which allows assessment of tissue mechanical properties as well as imaging properties [13, 16].

The authors also will focus on the clinical implementation of OCT. The first most obvious approach is the use of OCT in arthroscopy. An OCT catheter has been recently developed that is small enough to be introduced through the flush port of a standard arthroscope, which can be used to assess, for example, the ACL. In addition, an OCT imaging needle has been developed that has a cross-sectional diameter less than 400 μm [11]. This needle is a minimally invasive device, which can be introduced directly into the joint space to assess the tendon/ligament without the need for tissue removal or significant collateral tissue injury. This could allow the direct assessment of the tissue with minimal destruction.

This study demonstrates the ability of polarization-sensitive OCT to define the highly organized nature of collagen in tendons and ligaments. The integrity of collagen is an important indicator of structural stability and pathologic state. Therefore, the ability of OCT to assess collagen could be a powerful diagnostic tool in assessing tendon and ligament properties in the clinical setting.

Acknowledgments This research is supported in part by the National Institutes of Health, Contract NIH R01 HL63953-01 (MEB), NIH R01 AR46996-01 (MEB), NIH-9-R01-EY11289-14 (JGF), NIH-1-R01-CA75289-04 (JGF); the Medical Free Electron Laser Program, Office of Naval Research Contract Grant F49620-01-1-0186 (JGF and MEB); and the National Institutes of Health, Contracts 1R01AR44812-01 (MEB), NIH-1-R29-HL55686-01A1 (MEB), NIH-R01-AR44812-02 (MEB), R01 HL55686-07 (MEB), and R01EB000419-01A1 (MEB).

References

1. Astrom M and Rausing A (1995) Chronic Achilles tendinopathy: A survey of surgical and histopathologic findings. *Clin Orthop* 316:151–164
2. Azangwe G, Mathias KJ, Marshall D (2000) Macro and microscopic examination of the ruptured surfaces of anterior cruciate ligaments of rabbits. *J Bone Joint Surg [Br]* 82:450–456
3. Boppart SA, Bouma BE, Pitris C, et al (1998) In vivo subcellular optical coherence tomography imaging in *Xenopus laevis*: Implications for the early diagnosis of neoplasms. *Nature-Medicine* 4:861–865
4. Brezinski ME, Tearney GJ, Bouma BE, et al (1996) Optical coherence tomography for optical biopsy: properties and demonstration of vascular pathology. *Circulation* 93:1206–1213
5. Drexler W, Stamper DL, Jesser C, et al (2001) Correlation of collagen organization with polarization sensitive imaging in cartilage: Implications for osteoarthritis. *J Rheumatol* 28:1311–1318
6. Herrmann J, Pitris C, Bouma BE, et al (1999) High resolution imaging of normal and osteoarthritic cartilage with optical coherence tomography. *J Rheumatol* 26:627–635
7. Huang D, Swanson EA, Lin CP, et al (1991) Optical coherence tomography. *Science* 254:1178–1181

8. Junqueira L, Figueiredo M, Torloni H, Montes G (1986) Differential histologic diagnosis of osteoid. *J Pathology* 148:189–196
9. Kartus J, Magnusson L, Stener S, et al (1999) Complications following arthroscopic anterior cruciate ligament reconstruction: A 2–5-year follow-up of 604 patients with special emphasis on anterior knee pain. *Knee Surg Sports Traumatol Arthroscopy* 7:2–8
10. Kartus J, Movin T, Papadogiannakis N, et al (2000) A radiographic and histologic evaluation of the patellar tendon after harvesting its central third. *Am J Sports Med* 28:218–226
11. Li X, Chudoba C, Ko TH, et al (2000) Imaging needle for optical coherence tomography. *Optics Letters* 25:1520–1522
12. Movin T, Gad A, Reinholt FP, et al (1997) Tendon pathology in long-standing achillodynia: Biopsy findings in 40 patients. *Acta Orthop Scand* 68:1701–1775
13. Schmitt J. (1998) OCT elastography: Imaging microscopic deformation and strain of tissue. *Optics Express* 6:199–211
14. Sweat F, Puchtler H, Rosenthal S (1964) Sirius red F3BA as a stain for connective tissue. *Archives of Pathology* 78:69–72
15. Tearney GJ, Brezinski ME, Bouma BE et al (1997) In vivo endoscopic optical biopsy with optical coherence tomography. *Science* 276:2037–2039
16. Van der Steen A, de Korte CL, Cespedes EI (1998) Intravascular ultrasound elastography. *Ultraschall in Med* 19: 196–201
17. Wojtys EM, Huston LJ, Lindenfeld TN, et al (1998) Association between the menstrual cycle and anterior cruciate ligament injuries in female athletes. *Am J Sports Med* 26: 614–619