Ex Vivo Imaging of Chronic Total Occlusions Using Forward-Looking Optical Coherence Tomography

Nigel R. Munce, MSc,1* Victor X.D. Yang, MD, PhD,1,2,5 Beau A. Standish,1 Beiping Qiang, MD, PhD,3 Jagdish Butany, MD,6 Brian K. Courtney, MD,4 John J. Graham, MD,4,5 Alexander J. Dick, MD, FACC,4,5 Bradley H. Strauss, MD, PhD, FACC,3 Graham A. Wright, PhD,1,5 and I. Alex Vitkin, PhD1,2,7

1Department of Medical Biophysics, University of Toronto, Toronto, Canada
2Division of Biophysics and Bioimaging, Ontario Cancer Institute, University of Toronto, Toronto, Canada
3Roy and Ann Foss Interventional Cardiology Research Program, Terrence Donnelly Heart Centre, St Michael’s Hospital, University of Toronto, Toronto, Canada
4Division of Cardiology, Sunnybrook Health Science Centre, University of Toronto, Toronto, Canada
5Imaging Research Program, Sunnybrook Health Science Centre, University of Toronto, Toronto, Canada
6Department of Laboratory Medicine and Pathobiology; and Division of Pathology, University Health Network, University of Toronto, Toronto, Canada
7Department of Radiation Oncology, University of Toronto, Toronto, Canada

Background and Objectives: Percutaneous coronary interventions (PCI) of chronic total occlusions (CTOs) of arteries are more challenging lesions to treat with angioplasty and stenting than stenotic vessels due primarily to the difficulty in guiding the wire across the lesion. Angiography alone is unable to differentiate between the occluded lumen and the vessel wall and to characterize the content of the occlusion. New technologies to aid in interventional guidance are therefore highly desirable. We sought to evaluate tissue characterization in arterial (CTOs) by imaging ex vivo peripheral arterial samples with optical coherence tomography (OCT).

Study Design/Materials and Methods: Ex vivo arterial samples were obtained from patients undergoing peripheral limb amputation. Samples were imaged in an en face orientation using an OCT system, enabling sequential acquisition of longitudinal images and volumetric reconstruction of cross-sectional views of the occluded arteries. Histology was performed for comparison.

Results: OCT imaging reliably differentiated between the occluded lumen and the underlying arterial wall in peripheral CTOs. OCT correctly identified tissue composition within the CTO, such as the presence of collagen and calcium and was also able to identify intraluminal microchannels.


Key words: intravascular imaging; arterial disease; interventional cardiology; optical coherence tomography; chronic total occlusions

INTRODUCTION

Chronic total occlusions (CTO) of coronary and peripheral arteries are generally defined as occluded arteries of 3 months duration or longer [1]. Totally occluded arteries are observed in as many as one-third of all X-ray angiograms [2]. The observation of a CTO under X-ray angiography is the most common reason for referral to bypass surgery as opposed to minimally invasive percutaneous approaches [3]. Percutaneous interventions of coronary and peripheral artery CTOs have only limited success rates (approximately 75% in coronary lesions [4]) due to the inability of the operator to easily direct a guidewire through the occluded lumen without dissecting the adjacent arterial wall. A limited number of histological studies have shown that the occluded lumen of a CTO is a complex lesion containing variable amounts of collagen, lipids, calcification, and intraluminal microchannels [5,6]. The border between lumen and the underlying arterial wall cannot be visualized by contrast angiography, leading to the frequent occurrence of directing guidewires into a subintimal location and procedural failure, even possibly vessel perforation. Recently, we have suggested that intraluminal microchannels, 100–200 μm in diameter, which cannot be identified by current imaging techniques, may play a role in predicting the ease with which the occlusion can be crossed with a guidewire [7]. There is thus a need for investigating new imaging modalities to aid in characterization and guidance of intervention in CTOs.
Optical coherence tomography (OCT) is an imaging modality that uses the interference of light reflected back from the tissue with light from a reference arm to form an image based on the depth-resolved reflectivity of the sample, to a depth of approximately 2 mm in tissue [8]. OCT’s axial resolution, on the order of 3–15 μm, has been shown to enable differentiation of arterial layers [9] and identification of various arterial pathologies [10,11]. Contrast in OCT is provided by both the intrinsic back-scattering properties of the different tissue types, as well as reflections from the different layers. In the intravascular forward-looking geometry, more amenable to interventional guidance, tissue interfaces are likely to be at an acute angle to the imaging direction. Thus contrast is mostly derived from the reflective properties of the tissue itself.

Recently, an optical coherence reflectrometry system, which utilized only single-line (one-dimensional) depth profiles of reflections from interfaces, was approved for use in CTOs. Initial studies suggested that this system was able to differentiate between deeper layers of the vessel wall and the occluded lumen [12–15]. However, this system does not provide images of vessel layer boundaries or identify tissue composition within each layer.

We have thus been examining the potential utility of a forward-looking OCT imaging geometry to provide detailed cross-sectional images of the arterial wall and reliably differentiate tissue layers and identify specific tissue composition. We now report on our first ex-vivo experience with OCT imaging of human peripheral CTOs.

MATERIALS AND METHODS

Sample Preparation

Twenty-two samples of peripheral CTOs were obtained from below knee amputated limbs from 14 patients with peripheral artery disease, with informed consent under protocols approved by the hospital research ethics committee. Angiograms were obtained prior to amputation to identify the area of occlusion. The diseased arteries were dissected out of the limbs following amputation and stored in phosphate-buffered saline at 4°C. The arteries were cut into 5-mm sections. The maximum time between amputation and imaging was 1 week.

OCT Imaging

Arterial samples were imaged using a previously described time-domain OCT system [16]. The geometry of the scanning is illustrated in Figure 1. OCT images are presented on a log-based gray scale with scale bars of 1 mm appearing in red in the figures. This system employs a broadband low coherence source with a polarized output of 18 mW at a center wavelength of 1.3 μm with a bandwidth of Δλ = 63 nm, yielding a coherence length (axial resolution) of ~10 μm in tissue. The reference arm consists of a rapid scanning optical delay line and a phase modulator. The sample arm consists of a single-mode fiber capped with a ball-lens, mounted on a three-dimensional computer-controlled micro-positioning stage, yielding a spot size (lateral resolution) of ~20 μm.

Samples were attached to a piece of Styrofoam in order to preserve their orientation for later histological analysis. The specimen was oriented with the vessel axis parallel to the imaging fiber in order to approximate a forward-viewing intravascular imaging geometry. Lateral scanning combined with coherence gate depth scanning yielded a two-dimensional subsurface longitudinally-oriented OCT image, acquired at a rate of one frame per second. The probe was then translated across the sample in the orthogonal lateral direction in 10 μm increments, to yield a series of approximately 300 adjacent longitudinal slices acquired in 5 minutes that were then used for three-dimensional reconstruction and visualization.

For off-line three-dimensional visualization and reconstruction, OCT images were downloaded into Amira Visualization software (Mercury Computer Systems, Berlin, Germany). This software was used to reconstruct perpendicular (cross sectional) views of the arterial sample, by converting the set of adjacent two-dimensional longitudinal images into a three dimensional volume which

![Fig. 1. Schematic of the OCT probe—Arterial sample arrangement used for OCT image acquisition. (Figure can be viewed in color online via www.interscience.wiley.com.)](image-url)
could then be viewed in arbitrary orientations/projections. Reconstruction time was approximately 10 seconds. Brown lines are shown through each OCT image in the figures to indicate the location of the corresponding orthogonal slice.

**Histology Processing**

Immediately following OCT imaging, arterial samples were placed in formalin and sent for histology. A cross-sectional slice was first obtained and then the sample was re-embedded and sectioned longitudinally. Longitudinal sections were taken every 100 μm to ensure similar views in both histology and OCT imaging. Sections were stained with elastin trichrome to identify elastic tissue (black), muscle and blood (red), and collagen (blue). Calcification was identified with Von Kossa’s stain as black. Lipid was identified with Oil Red O in frozen sections (showing lipid as red). Histology slides were scanned under a white light slide scanner (Aperio Technologies, Vista, CA) to allow for high resolution (20 μm2) large field-of-view histology images.

**RESULTS**

CTOs obtained and scanned could be generalized into several principal types based on histological appearance:

1. Extensively calcified wall, dense collagen occupying the lumen (n = 6).
2. Microcalcifications within the lumen, embedded in collagen (n = 6).
3. Extensive smooth muscle cell infiltration with collagen in lumen (n = 4).
4. High lipid content in lumen (n = 2).
5. Dense collagen within lumen (n = 4).

In the OCT images, dense fibrotic tissue appeared bright, while highly cellular areas and looser connective tissue appeared darker. Such an occlusion is shown in Figure 2 where smooth muscle cells infiltrating the occluded lumen (OL) are seen as a dark region within the occlusion on the OCT images (Fig. 2a,b) and as a red stain within occluded lumen in the elastic trichrome histology (Fig. 2c,d). The muscular media in this case also appears as dark region (M) in both longitudinal (a) and reconstructed axial (b) OCT slices.

Figure 3 shows an occluded artery with an occluded lumen composed of mostly collagen that show up as uniformly scattering region under OCT (Fig. 3a,b). Moderate calcium deposits (C) within the media layers are seen under histology (Fig. 3c,d).

Figure 4 presents a very chronic, heavily calcified occlusion in which intramural calcification appeared as superficially reflective, signal poor regions within the arterial wall as shown in the region labeled C in the longitudinal OCT image in Figure 4a. Representative histology is shown in Figures 4c,d that illustrate large areas of “fallout” indicative of large pieces of calcium.

An example of an occluded artery with extensive intraluminal microcalcifications embedded within the...
collagen matrix is shown in Figure 5. These microcalcifications were seen as highly reflective spots under OCT within the CTO that greatly attenuate the OCT signal with depth as shown in Figures 5a,d in the region labeled C.

A CTO with large lipid deposits is presented in Figure 6. Regions of lipids (L) were observed as signal poor spaces within the CTO as shown in OCT images in Figure 6a,b and confirmed using Oil Red stain shown in Figure 6c.

Intraluminal microchannels within the occlusion were identified in most OCT images as small crevices on the longitudinal slices and holes on the cross sectional slices; that were confirmed by histology. The appearance of the different potential components of CTOs under OCT is summarized in Table 1.

**DISCUSSION**

This is the first study to report on ex vivo imaging and characterization of CTOs using OCT. We have been able to use multiple longitudinal OCT slices to generate cross sectional images of occluded arteries. In all cases, these reconstructed axial views exhibited significantly more information than the original longitudinal slices. The calculated reconstructed cross sectional slices were crucial for reliably differentiating the arterial wall layers (occluded lumen, media, and adventitia) and identifying specific tissue composition within the occluded lumen and the media.

**Identification of the Vessel Wall**

Due to its high collagen content, the occluded lumen of the vessels typically had a higher back-scattering signal than the surrounding medial layers allowing for identification of the occlusion. Cases in which the media appeared fibrotic and hence bright on OCT (as in Fig. 5a) still displayed small muscular regions that maintained a dark OCT signal. This variation in intensity from the media illustrates the necessity of imaging in order to identify the different arterial layers. Additionally, the advential layer could be identified in most cases, except those in which severe intramural calcification obscured the boundary between the media and adventitial layers as is seen in Figure 4.

**Identification of Specific Tissue Characteristics**

This is the first demonstration of OCT’s capacity to visualize microchannels in CTOs. In all cases, endoluminal microchannels, greater than 50 μm in size, could be accurately identified within the occluded lumen by OCT on reconstructed axial slices. Initial imaging studies using μCT have shown the ability to perfuse these channels with...
radio-opaque casting polymers [7] suggesting that clearing these channels of blood with a saline flush, as would be required for in vivo imaging, is possible. In combination with clearing of the blood field in a CTO, microchannels may provide greater OCT imaging depth than one would normally expect if the occlusion were completely solid. This increase in depth range is an ideal application for new frequency-domain OCT systems that could provide up to 4 mm imaging through a clarified straight microchannel [17].

In this study, intramural calcification appeared as highly reflective regions within the wall of the artery with a signal poor region below it. This appearance is different to that reported in previous OCT arterial studies [10,11] using side-viewing OCT geometries that reported a clearly defined signal poor region. We attribute this difference to the segmental nature of the samples scanned in an en face geometry in this study as opposed to a side-viewing probe. In the side-viewing case, calcification is viewed through the intimal layer of the artery and therefore the reflection is not as pronounced as it is when viewing an air-calcium interface as presented here.

The OCT appearance of lipid, smooth muscle cell, fibrotic regions, and microcalcifications reported in this study agrees with previous reports on these components studied in vessel wall imaging [10,11,18]. The identification of lipid by OCT was, however, often obscured due to its co-localization with microcalcifications in the CTO.

Thrombus within or around the occlusion was occasionally seen as a bright signal lining the microchannel(s) of the CTO. Studies by others [19] suggest OCT’s ability to identify and differentiate between red and white thrombus within the coronary arteries. Whether OCT can be used to determine the extent of organization within thrombus remains, however, a subject of future work.

**Significance of Imaging CTOs for Tissue Boundaries and Specific Tissue Composition**

Advanced CTO imaging techniques that are additive to contrast angiography remain a highly desired but unrealized goal. Success rates in CTOs, both peripheral and coronary, are much lower than stenotic but non-fully occluded lesions, in part due to inadequate visualization of the occluded segment. Additionally, the lack of imaging modalities capable of discerning the composition of total occlusions has resulted in a poor understanding of the role of each specific tissue component in procedural success or failure. For example, several studies have offered differing views on the role of calcification as an adverse predictive factor for successful CTO recanalization [20,21]. However,
contrast angiography is limited in identifying intramural calcifications and their precise location within the wall. It is likely that calcification restricted to deep arterial wall layers will have a different effect than intraluminal calcifications. Other components of CTOs, particularly collagen, smooth muscle cells and lipid, which can currently only be assessed through histological means, may also portend different success rates. New therapies, such as enzyme-mediated collagen degradation [22], may be better-suited for specific lesion pathologies that cannot be currently identified nor differentiated by contrast angiography but are within the diagnostic ability of OCT. Recently, we have also suggested that intraluminal microchannels are an important predictor of lesion crossing and a potential target for angiogenic CTO therapies [7]. The identification of these microchannels by imaging modalities such as OCT could help clinicians select favorable CTOs for treatment, guide therapy during percutaneous interventions, and also assess the effects of angiogenic therapeutic approaches. Conversely, large lipid deposits may identify more complex CTOs with high likelihood of distal embolization. Thus, the identification of these lipid deposits within the CTO could alter the decision to attempt therapy on a CTO to avoid this risk or potentially allow the interventionalist to try to avoid the large lipid core. While more work is required to identify OCT's sensitivity and specificity in identifying these pathological features, this study suggests that OCT's ability to provide a detailed subsurface image of an occluded artery may identify an optimal interventional path and assess the variation in the arterial wall integrity across a large region of the artery.

**In Vivo Implementation**

Practical in vivo intravascular forward-viewing OCT imaging will require several improvements to the probe design used here. The probe must be flexible and self-contained (no exposed moving parts) with a diameter of 2 mm or less in order to facilitate access to the coronary arteries. Forward-viewing OCT probes [23–26], typically have long rigid segments (> 2 cm) at the distal end that would be unsuitable for use within tortuous vasculature. The probe must also image a wide viewing area in order to visualize the entire occlusion. Designs involving scanning a fiber optic across a GRIN lens would be amenable to such requirements; however miniaturization of such probes remains an ongoing research area. In order to obtain three dimensional images in vivo, similar to the ones presented here, in vivo, such scanning probes could be simultaneously rotated using a torque cable. Frequency domain OCT systems comparable to those reported in the literature [27], would be required to ensure clinically acceptable volumetric imaging times on the order of a second or less. Such high frame-rate systems may permit imaging within the time window created by a saline flush alone, without the
need for an occluding balloon. Such a saline flush would be necessary in vivo as blood would severely degrade the image quality.

The intravascular image created in such a system would likely only visualize the media and adventitia layers in the larger scan angles of the image due to the limited imaging depth of OCT. Despite this limitation, the ability to resolve the different arterial layers as well identify constituents of the occlusion and identify microchannels would add a valuable tool to the interventionist’s arsenal.

### TABLE 1. OCT Signal Characteristics of CTO Constituents

<table>
<thead>
<tr>
<th>CTO component</th>
<th>OCT appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perivascular tissue (loose connective tissue surrounding the artery)</td>
<td>Dark border surrounding the artery</td>
</tr>
<tr>
<td>Adventitia</td>
<td>Signal rich peripheral border of the vessel</td>
</tr>
<tr>
<td>Media</td>
<td>Signal rich in significant fibrosis or signal poor when it maintains its muscular nature</td>
</tr>
<tr>
<td>Collagen within the lumen</td>
<td>Uniformly back-scattering region. Denser collagen has a higher back-scattering signal (Figs. 3 and 4)</td>
</tr>
<tr>
<td>Smooth muscle cells within the lumen</td>
<td>Dark regions within the collagen matrix of the CTO. Not very reflective (Fig. 2)</td>
</tr>
<tr>
<td>Intraluminal microchannels</td>
<td>Fine cracks within the CTO. Residual blood shows as a bright reflective lining Lightly scattering in large pools; Can be identified also as small “segments” (Fig. 6)</td>
</tr>
<tr>
<td>Lipid</td>
<td>Lightly scattering in large pools; Can be identified also as small “segments” (Fig. 6)</td>
</tr>
<tr>
<td>Microcalcifications within the CTO</td>
<td>Highly reflective dots. When abundant, they create shadows (Fig. 5)</td>
</tr>
<tr>
<td>Intramural calcium</td>
<td>Highly reflective on the surface; otherwise signal poor (Fig. 4)</td>
</tr>
</tbody>
</table>

Fig. 6. OCT images of an occluded anterior tibial artery demonstrating high lipid content are shown in (a) and (b). A small central microchannel (MC) is seen in both reconstructed cross sectional OCT slices and histology. Lipid deposition, labeled L, is seen both within the collagen matrix of the CTO as well as accumulation around the central microchannel on the Oil Red O histology shown in (c). These regions are seen as weakly scattering regions in the OCT images. Lipid deposition around the central microchannel appears as small segmental deposits seen in the longitudinal OCT image shown in (a). Areas of the lumen containing a high collagen content once again appear as bright under the OCT images. Histology is Oil Red O in (c) and Elastin Trichrome (d). Bars = 1 mm. [Figure can be viewed in color online via www.interscience.wiley.com.]
ACKNOWLEDGMENTS

The authors acknowledge the work of the special histology lab at Mt. Sinai Hospital, as well as the help of Peter Faure in the department of pathology at Toronto General Hospital. Support for this project was provided by the Canadian Foundation for Innovation, the Ontario Centers of Excellence, Photonics Research Ontario, as well as the Canadian Institutes of Health Research.

REFERENCES


