Characterization of Human Atherosclerosis by Optical Coherence Tomography

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Background—High-resolution visualization of atherosclerotic plaque morphology may be essential for identifying coronary plaques that cause acute coronary events. Optical coherence tomography (OCT) is an intravascular imaging modality capable of providing cross-sectional images of tissue with a resolution of 10 μm. To date, OCT imaging has not been investigated in sufficient detail to assess its accuracy for characterizing atherosclerotic plaques. The aim of this study was to establish objective OCT image criteria for atherosclerotic plaque characterization in vitro.

Methods and Results—OCT images of 357 (diseased) atherosclerotic arterial segments obtained at autopsy were correlated with histology. OCT image criteria for 3 types of plaque were formulated by analysis of a subset (n=50) of arterial segments. OCT images of fibrous plaques were characterized by homogeneous, signal-rich regions; fibrocalcific plaques by well-delineated, signal-poor regions with sharp borders; and lipid-rich plaques by signal-poor regions with diffuse borders. Independent validation of these criteria by 2 OCT readers for the remaining segments (n=307) demonstrated a sensitivity and specificity ranging from 71% to 79% and 97% to 98% for fibrous plaques, 95% to 96% and 97% for fibrocalcific plaques, and 90% to 94% and 90% to 92% for lipid-rich plaques, respectively (overall agreement, κ=0.83 to 0.84). The interobserver and intraobserver reliabilities of OCT assessment were high (κ values of 0.88 and 0.91, respectively).

Conclusions—Objective OCT criteria are highly sensitive and specific for characterizing different types of atherosclerotic plaques. These results represent an important step in validating this new intravascular imaging modality and will provide a basis for the interpretation of intracoronary OCT images obtained from patients. (Circulation. 2002;106:1640-1645.)

Key Words: atherosclerosis ■ catheters ■ imaging ■ tomography ■ plaque

Spontaneous rupture of atherosclerotic plaques with subsequent thrombosis is the most frequent underlying cause of acute coronary events and sudden death.1,2 Autopsy studies have identified several histological characteristics of these vulnerable plaques: (1) a large lipid pool, (2) a thin fibrous cap (<65 μm), and (3) activated macrophages near the fibrous cap.3,4 A variety of morphological imaging technologies are currently under investigation to detect vulnerable plaques with the hope of guiding patient management and monitoring response to intervention.

Intravascular optical coherence tomography (OCT) is a recently developed optical imaging technique that provides high-resolution cross-sectional images of tissue in situ.5,6 The typical OCT image has a homaxial resolution of 10 μm and a lateral resolution of 20 μm, which is ~10 times higher than that of any clinically available diagnostic imaging modality. Experiments correlating a limited number of excised coronary and aortic specimens with histology have demonstrated that OCT is capable of resolving microstructural features of atherosclerotic plaques.7-9 Animal studies performed in vivo have demonstrated the use of an intravascular OCT catheter for directly imaging normal rabbit aortas10 and swine coronary arteries.11 We recently demonstrated the safety and feasibility of intracoronary OCT imaging in patients.9,12 The unique capability of OCT to resolve micrometer-scale features of atherosclerosis makes this new imaging modality an attractive means for identifying features of coronary plaques at risk for rupture. To date, however, this technology has not been investigated in sufficient detail to assess its accuracy for characterizing atherosclerotic plaques. The purpose of the present study was to establish OCT criteria for characterizing atherosclerotic plaques in vitro by correlating OCT images with histology.
Methods

Specimens
We examined 357 grossly diseased arterial segments (162 aortas, 105 carotid bulbs, and 90 coronary arteries) from 90 randomly selected cadavers (48 male and 42 female, mean age 74.5±13.2 years). Of these patients, 48 had symptomatic cardiovascular disease (53%). The harvested arteries were stored immediately in PBS at 4°C. The time between death and OCT imaging did not exceed 72 hours. The experimental protocol was approved by the Institutional Review Board at the Massachusetts General Hospital.

OCT Imaging Studies
The OCT system used in the present study has been described previously. OCT images were acquired at 4 frames per second (500 angular pixels×250 radial pixels), displayed with a gray-scale lookup table, and digitally archived. The optical source used in this experiment had a center wavelength of 1310 nm and a bandwidth of 65 nm, providing an axial resolution of ~10 μm in tissue.

Before OCT imaging, arteries were warmed to 37°C in PBS. Each carotid bulb and aorta was opened and imaged with the luminal surface exposed by use of a computer-controlled galvanometer to scan the infrared beam across the specimen. Coronary arteries were imaged with 3.2F OCT catheters. The transverse resolution for both the galvanometer and catheter probes was 25 μm.

The position of the interrogating beam on the tissue was monitored by a visible light-aiming beam (laser diode, 635 nm) that was coincident with the infrared beam. Precise registration of OCT and histology was achieved by application of ink marks (Triangle Biomedical Sciences) to the vessels at the imaging site, such that each OCT image and corresponding histological section contained visually recognizable reference points.

Histopathology
After imaging, the tissue was processed routinely. Arterial segments were fixed in 10% formalin (Fisher Scientific) for ≥48 hours. Arteries with substantial calcification were decalcified (Cal-EX, Fisher Scientific) before further processing. They were then processed for standard paraffin embedding. Sections 4 μm thick were cut at the marked imaging sites and stained with hematoxylin and eosin (H&E) or Movat’s pentachrome.

Correlation Between OCT Images and Histopathology
We obtained 357 pairs of OCT images and correlating histological sections using the ink marks as points of reference. Each OCT/histology pair was randomly assigned a unique identification number and separated into 2 sets, a “training set” (n=50) and a “validation set” (n=307).

The training set was used to establish OCT image criteria for the 3 types of histological plaques (fibrous, fibrocalcific, and lipid-rich). The criteria were determined by assessing morphological features observed by OCT and histology and correlating their size, shape, and location using the ink marks as points of reference.

Subsequently, the OCT image criteria were prospectively applied to the validation set by 2 OCT observers blinded to the histopathologic diagnosis (OCT reader 1, C.R.K., and OCT reader 2, B.E.B.). Two pathologists (pathologist 1, S.L.H., and pathologist 2, H.T.A.) blinded to the OCT results classified all of the plaques in the validation set as fibrous, fibrocalcific, or lipid rich. Both pathologists and OCT readers were instructed to provide a single diagnosis for each slide or image. For all cases in which there was an initial disagreement between the 2 pathologists, pathologist 1 and pathologist 2 reread the slides and reached a consensus diagnosis. All observers were blinded to clinical information. To assess the OCT intraobserver variability, one OCT observer (OCT reader 1) reapplied the same OCT criteria to the validation set 1 month after the first reading.

Statistics
The accuracy of OCT for characterizing plaque type was calculated by application of OCT criteria to the validation set, with histopathologic consensus diagnosis as the “gold standard.” The degree of agreement between histopathologic diagnosis and the results obtained by the OCT readers and the OCT interobserver and intraobserver variability were quantified by the κ test of concordance.

Interobserver variability of the histopathologic diagnosis was also quantified. All continuous variables are expressed as mean±SD.

Results

OCT Criteria
Examination of the training set revealed distinct image features for each plaque type. Images of histologically confirmed fibrous plaques (n=11) exhibited homogeneous, highly backscattering (ie, signal-rich) plaque interiors devoid of OCT signal-poor regions (Figure 1). In contrast to images of fibrous plaques, OCT of histologically confirmed fibrocalcific plaques (n=27) revealed signal-poor regions with sharply delineated upper and/or lower borders (Figure 2). Images of histologically confirmed lipid-rich plaques (n=12) showed diffusely bordered, signal-poor regions (lipid pools)
with overlying signal-rich bands, corresponding to fibrous caps (Figure 3).

Validation
Tables 1 and 2 summarize the performance of the OCT image criteria applied to the validation set. These OCT image criteria showed a high sensitivity and specificity for characterizing plaque type (Table 2). The overall agreement between the OCT image criteria and the consensus histopathologic diagnosis for both OCT readers was high ($\kappa=0.83$ to 0.84).

Reproducibility
The interobserver and intraobserver agreements for characterization of plaque type by use of the OCT image criteria were also high ($\kappa=0.88$ and $\kappa=0.91$, respectively). The application of a higher threshold for identifying the presence of signal-poor regions by OCT reader 1 resulted in a lower sensitivity for lipid-rich regions (90% versus 94%) and a higher sensitivity for fibrous plaques (79% versus 71%), compared with OCT reader 2. The interobserver reliability of the initial histopathologic diagnosis of plaque type was $\kappa=0.83$.

Discussion
This is the first study to assess the accuracy of OCT for diagnosing plaque type. In a large series of autopsy specimens, we have developed objective OCT image criteria for differentiating distinct components of atherosclerotic tissue in vitro. In our analysis, these criteria achieved a high sensitivity and specificity for plaque characterization. High intraobserver and interobserver reliabilities were also demonstrated. These results represent an important step in validating this new intravascular imaging modality and will provide a basis for the interpretation of OCT images obtained in clinical studies.

Sources of Disagreement Between OCT and Histopathologic Diagnosis

The present study demonstrates that simple visual OCT criteria can be highly sensitive and specific for characterization of lipid-rich plaques. The accuracy of qualitative diagnostic criteria, however, is subject to the application of thresholds to delineate between distinct diagnostic entities. False-positive OCT diagnoses of lipid-rich plaques (n=20 and 23) often contained histological evidence of small amounts of lipid present within a predominantly fibrous plaque (Figure 4, A and B). These lesions, perceived as lipid-rich by OCT, were interpreted as fibrous plaque by histopathology, resulting in a relatively low sensitivity of the OCT criteria for diagnosing fibrous plaques (71% to 79%). In these cases, the disagreement between the OCT readers and the pathologists may represent the application of different
size thresholds for diagnosis. As a result, further modifications of these OCT criteria, such as the addition of a dimensional threshold for the signal-poor region, may be needed to better differentiate clinically relevant, large lipid pools from insignificant lipid accumulations.

Other sources of discrepancy between OCT and histological diagnosis could be attributed to the imaging penetration of OCT and the presence of heterogeneous plaques in the data set. False-negative diagnoses for lipid-rich plaques (n=7 and 4) could be attributed to the limited penetration depth (≈1 to 2 mm) of OCT, causing some thick-capped, large lipid pools (cap thickness >500 µm) to be misinterpreted as fibrous plaques (Figure 4, C and D). Fibrocalcific plaques misclassified by OCT (false-negative for fibrocalcific plaques, n=8 and 6) were typically complex plaques by histology, consisting of calcific nodules with surrounding fibrous tissue and lipid (Figure 4, E and F).

Study Limitations
One limitation of the present study is the use of cadaver specimens, because minor tissue changes may have occurred postmortem. The effect of specimen degradation over a period of 72 hours (the maximum time between death and OCT imaging in the present study) on arterial optical properties is not known. However, a recent feasibility study performed in patients9 has shown that OCT images of arterial plaques obtained in vivo demonstrate features similar to those identified in this work (Figure 5, A–C). Therefore, it is likely

| TABLE 2. Assessment of OCT Image Criteria for Plaque Characterization |
|-----------------------------|-----------------------------|-----------------------------|
| Histopathologic Diagnosis  | Sensitivity | Specificity | Positive Predictive Value | Negative Predictive Value |
| OCT reader 1               |              |              |                           |                           |
| Fibrous (n=77)             | 79 (68–88) | 97 (94–99) | 91 (82–97) | 93 (89–96) |
| Fibrocalcific (n=162)      | 95 (91–98) | 97 (92–99) | 97 (93–99) | 95 (90–98) |
| Lipid rich (n=68)          | 90 (80–96) | 92 (87–95) | 75 (64–84) | 97 (94–99) |
| OCT reader 2               |              |              |                           |                           |
| Fibrous (n=77)             | 71 (60–81) | 98 (96–100) | 93 (84–98) | 91 (87–94) |
| Fibrocalcific (n=162)      | 96 (92–99) | 97 (92–99) | 97 (93–99) | 96 (91–98) |
| Lipid rich (n=68)          | 94 (86–98) | 90 (86–94) | 74 (63–82) | 98 (95–100) |

Data are percentages. Numbers in parentheses are 95% confidence intervals.

Figure 4. A, OCT image of a carotid plaque containing small amounts of lipid (arrow) (false-positive for lipid-rich plaque). B, Corresponding histology (H&E; magnification ×20). C, OCT image of a lipid-rich aortic plaque containing a lipid pool (L) that is not clearly visualized because of limited penetration of light through cap (false-negative for lipid-rich plaque). D, Corresponding histology (H&E; magnification ×20). E, OCT image of a complex carotid plaque containing a significant amount of calcium (arrowheads) and a small amount of lipid (arrow) (false-negative for fibrocalcific plaque). F, Corresponding histology (H&E; magnification ×20). Bars=500 µm.

Figure 5. OCT images of coronary plaques and thrombus acquired from living human patients.9 A, From 11 o’clock to 1 o’clock positions, this OCT image demonstrates a layered morphology with intimal hyperplasia (see Figure 1). A plaque extending from 2 o’clock to 10 o’clock positions contains a homogeneous, signal-rich region, consistent with fibrous tissue (F, see Figure 1). B, This OCT image demonstrates well-delineated, signal-poor regions suggestive of calcifications (arrows, see Figure 2). C, This OCT image demonstrates a plaque containing a signal-poor region with diffuse borders (L), possibly representing a lipid pool (see Figure 3). D, This OCT image shows an irregular, homogenous tissue adherent to vessel surface, consistent with a thrombus (D, inset). Guidewire shadow artifacts (*); tick marks, 1 mm.
that the OCT criteria evaluated here will also be applicable to intracoronary OCT images obtained from patients.

Because the present study was designed to evaluate the potential of OCT to characterize plaques before rupture, lesions containing thrombus were not analyzed. Identification of thrombus by OCT is important for plaque characterization, especially in imaging of patients who have recently suffered a myocardial infarction. Postprocedural OCT images of presumed thrombi in patients have shown homogeneous, well-defined tissue adherent to the luminal surface and protruding into the vessel lumen (Figure 5D). Correlative studies comparing OCT images of thrombi at different stages of progression with histology should be conducted to provide additional criteria for plaque characterization after intimal disruption.

In the present study, lesions were characterized as either fibrous, fibrocalcific, or lipid-rich. Because atherosclerosis is a progressive disease, it is likely that many lesions may be heterogeneous and contain all of the above tissue components. Because both the pathologists and OCT readers were instructed to provide a single diagnosis for each slide or image, respectively, the analysis of heterogeneous plaques was not accommodated in the study design. As a result, many of the failures of the OCT image criteria to accurately determine plaque type may have been caused by plaque heterogeneity. A more sophisticated application of multiple OCT criteria should significantly improve OCT diagnosis of plaque constituents.

In addition to plaque characterization, measurement of fibrous cap thickness is of paramount importance for assessing plaque vulnerability in the atherosclerotic lesion. OCT images of lipid-rich plaques (Figures 3, 4A, and 5C) allow visualization of fibrous caps at a resolution not possible with any other arterial imaging modality. Although our tissue registration procedure was sufficiently precise (≈500 μm) for grossly correlating OCT imaging sites with histology, we found that it was often inadequate for correlating cap thickness. Future studies to correlate cap measurement with histology will be performed using a more precise registration method.

**Alternative Plaque Characterization Methods**

A summary of the results of comparable studies using different methods for differentiating lipid-rich plaques from fibrous/fibrocalcific plaques is presented in Table 3. MRI is one of the most promising noninvasive imaging modalities for identifying lipid-rich plaques. Several recent studies have shown that MRI is capable of characterizing atherosclerotic plaques with high sensitivities and specificities in vitro \(^\text{21,22}\) and in vivo (carotid arteries). \(^\text{17}\) However, the combination of cardiac and respiratory motion, small size, and location from the surface of the body may make MRI imaging of detailed coronary arterial structure in patients difficult. \(^\text{18}\)

A variety of catheter-based methods are currently under investigation for the characterization of atherosclerotic plaques. Intravascular ultrasound (IVUS) is a widely used imaging method in the field of interventional cardiology. A recent publication suggests a correlation between the presence of echoluent zones, presumably lipid pools, in IVUS images of coronary plaques and acute coronary events. \(^\text{19}\) Studies using IVUS to characterize plaque type, however, have shown that the discrimination between fibrous and lipid-rich tissue is difficult with this imaging modality. \(^\text{20–22}\)

Recent advances in IVUS technology, such as the use of 40-MHz transducers, \(^\text{23}\) radiofrequency signal analysis, \(^\text{24}\) integrated backscatter, \(^\text{25}\) and elastography, \(^\text{26}\) have improved the capability of IVUS for plaque characterization. Research performed with intravascular MRI (IVMRI) has demonstrated that IVMRI also is capable of differentiating plaque components. \(^\text{27}\) Nevertheless, the limited resolutions (≈50 to 100 μm) of both IVUS \(^\text{23,24,26}\) and IVMRI \(^\text{27}\) preclude accurate evaluation of relevant microstructural features, such as thin fibrous caps.

The ability of light to provide spectroscopic information relating to plaque composition has motivated researchers to pursue a variety of optical techniques for plaque identification. Studies investigating the use of angiography for plaque characterization have shown that the gross appearance of plaques corresponds to histopathologic findings. \(^\text{28–29}\) By use of the spectrum of light scattered from within the vessel wall, experiments investigating both diffuse-reflectance near-infrared spectroscopy and Raman spectroscopy have demonstrated accurate plaque characterization in vitro. \(^\text{30–32}\) On the basis of the hypothesis that local inflammation within vulnerable plaques may lead to local elevations in temperature, studies have recently been conducted using a temperature-sensing catheter. \(^\text{33,34}\) Experiments performed in patients have indicated that both temperature heterogeneity and the temperature difference between atherosclerotic plaque and healthy vessel walls increase with disease severity. \(^\text{34}\) In addition to providing compositional information, light can also be used to image tissue structure in situ at a higher resolution than other conventional imaging modalities. Structural optical imaging methods, such as OCT, allow visualization of microscopic features that correlate well with traditional histopathology and as a result, provide a reliable morphological complement to compositional methods for characterizing atherosclerosis.

**Conclusions**

The characterization of atherosclerotic plaques is assuming greater importance in determining the risk of cardiovascular events and may be needed to guide local intervention in the

**TABLE 3. Comparison of Methods for Differentiating Lipid-Rich Plaques From Fibrous/Fibrocalcific Plaques in Human Arterial Specimens In Vitro**

<table>
<thead>
<tr>
<th>Study Subjects†</th>
<th>Sensitivity, %</th>
<th>Specificity, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRI (2 T) (^\text{17})</td>
<td>67 (19)</td>
<td>74</td>
</tr>
<tr>
<td>IVUS (40 MHz) (^\text{25})</td>
<td>122 (29)</td>
<td>65</td>
</tr>
<tr>
<td>NIR Spectroscopy (^\text{33})</td>
<td>99 (39)</td>
<td>90</td>
</tr>
<tr>
<td>OCT</td>
<td>307 (68)</td>
<td>90–94</td>
</tr>
</tbody>
</table>

*Studies using 1 diagnosis per specimen with reported sensitivities and specificities are included in this table.

†Numbers in parentheses indicate number of lipid pools by histology.

NIR indicates near-infrared.
future. The present study shows that by using objective OCT image criteria, atherosclerotic plaques can be discriminated in vitro with a high degree of sensitivity and specificity. The unique capability of OCT to accurately characterize plaque components suggests that this new technique may hold promise for identifying morphological features of coronary plaques at risk for rupture.

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References

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