Quantification of Macrophage Content in Atherosclerotic Plaques by Optical Coherence Tomography

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**Background**—Macrophage degradation of fibrous cap matrix is an important contributor to atherosclerotic plaque instability. An imaging technology capable of identifying macrophages in patients could provide valuable information for assessing plaque vulnerability. Optical coherence tomography (OCT) is a new intravascular imaging modality that allows cross-sectional imaging of tissue with a resolution of $\approx 10 \text{ \mu m}$. The aim of this study was to investigate the use of OCT for identifying macrophages in fibrous caps.

**Methods and Results**—OCT images of 26 lipid-rich atherosclerotic arterial segments obtained at autopsy were correlated with histology. Cap macrophage density was quantified morphometrically by immunoperoxidase staining with CD68 and smooth muscle actin and compared with the standard deviation of the OCT signal intensity at corresponding locations. There was a high degree of positive correlation between OCT and histological measurements of fibrous cap macrophage density ($r=0.84, P<0.0001$) and a negative correlation between OCT and histological measurements of smooth muscle actin density ($r=-0.56, P<0.005$). A range of OCT signal standard deviation thresholds (6.15% to 6.35%) yielded 100% sensitivity and specificity for identifying caps containing $>10\%$ CD68 staining.

**Conclusions**—The high contrast and resolution of OCT enables the quantification of macrophages within fibrous caps. The unique capabilities of OCT for fibrous cap characterization suggest that this technology may be well suited for identifying vulnerable plaques in patients. (*Circulation. 2003;107:113-119.*)

**Key Words:** atherosclerosis ▪ catheters ▪ imaging ▪ tomography ▪ plaque

Cellularity of fibrous caps of atherosclerotic plaque, manifested by the infiltration of macrophages (average size, 20 to 50 $\mu m$), is thought to weaken the structural integrity of the cap and predispose plaques to rupture. Macrophages and other plaque-related cells produce proteolytic enzymes, such as matrix metalloproteinases, that digest extracellular matrix and compromise the integrity of the fibrous cap. Activated macrophages are strongly colocalized with local thrombi in patients who have died of acute myocardial infarction and are more frequently demonstrated in coronary artery specimens obtained from patients suffering from acute coronary syndromes compared with patients with stable angina. This evidence suggests that an imaging technology capable of identifying macrophages in patients would provide valuable information for assessing the likelihood of plaque rupture.

Intravascular optical coherence tomography (OCT) is a recently developed optical imaging technique that provides high-resolution, cross-sectional images of tissue in situ. The resolution of OCT, $\approx 10 \text{ \mu m}$, is appropriate for measuring the cap thickness ($<100 \text{ \mu m}$) that is characteristic of a vulnerable plaque. Previous studies have demonstrated the visualization of microstructural features in atherosclerotic plaques with OCT. Results from intracoronary OCT, recently performed in patients, have shown an improved capability for characterizing plaque microstructure compared with intravascular ultrasound. To date, however, the use of OCT for characterizing the cellular constituents of fibrous caps has not been investigated. The purpose of this study was to evaluate the potential of OCT for identifying macrophages in fibrous caps of atherosclerotic plaques.

**Methods**

**Specimens**
A total of 26 lipid-rich atherosclerotic arterial plaques (19 aortas and 7 carotid bulbs) were obtained from 17 randomly selected cadavers (10 male and 7 female, mean age 73.2±15.2 years) to quantify macrophage content. The harvested arteries were stored immediately.

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in PBS at 4°C. The time between death and OCT imaging did not exceed 72 hours. The Institutional Review Board at the Massachusetts General Hospital approved the experimental protocol.

OCT Imaging Studies
The OCT system used in the present study has been previously described. OCT images were acquired at 4 frames per second (500 angular pixels × 250 radial pixels), displayed with an inverse 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 70 nm, providing an axial resolution of ~10 μm in tissue. The transverse resolution, determined by the spot size of the sample arm beam, was 25 μm.

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. 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 accomplished by using a 26-gauge needle to apply ink marks (Triangle Biomedical Sciences) at the imaging site, OCT and histology was performed by using a 26-gauge needle to apply ink marks (Triangle Biomedical Sciences) at the imaging site.

Staining
After imaging, the tissue was processed in a routine fashion. Arterial segments were fixed in 10% formalin (Fisher Scientific) for at least 48 hours. Arteries with substantial calcification were decalcified (Cal-EX, Fisher Scientific) before standard paraffin embedding. Four-micron sections were cut at the marked imaging sites and stained with H&E and Masson’s trichrome (Cal-EX, Fisher Scientific) before standard paraffin embedding.

Morphometric Analysis

Macrophage Content Measurement
With the use of both digitized histology and OCT, measurements of macrophage density were obtained using a 500×125-μm (lateral x axial) region of interest (ROI), located in the center of the plaque (Figure 1). For caps having a thickness <125 μm, the depth of the ROI was matched to the cap thickness. In this study, tissue present within the ROI did not demonstrate histological evidence of either calcium or cholesterol crystals.

OCT measures the intensity of light returning from within a sample. Samples having a higher heterogeneity of optical index of refraction exhibit stronger optical scattering and therefore a stronger OCT signal. If the characteristic size scale of the index of refraction heterogeneity is larger than the resolution, then the OCT signal will have a larger variance. Previous research conducted to measure the optical properties of human tissue has shown that the refractive index of lipid and collagen is significantly different. These results suggest that caps containing macrophages should have multiple strong back reflections, resulting in a relatively high OCT signal variance. With standard image processing methods, the variance, σ², within the ROI of an OCT image can be represented by the following:

\[
\sigma^2 = \frac{1}{N-1} \sum_{ROIwidth} \sum_{ROIheight} (S(x,y) - \bar{S})^2,
\]

where N is the number of pixels in the ROI, ROIwidth is the width of the ROI, ROIheight is the height of the ROI, S(x,y) is the OCT signal as a function of x and y locations within the ROI, and \( \bar{S} \) is the average OCT signal within the ROI.

OCT images contain tissue back-reflection information that spans a large dynamic range (100 dB or 10 orders of magnitude). The dynamic range of OCT is too high to be displayed on a standard monitor, which may have a dynamic range of only 2 to 3 orders of magnitude. As a result, the signal range of most OCT images is compressed by taking the base 10 logarithm of the OCT image before display. Although taking the logarithm of the OCT image data enables convenient image display, compression of the data range in this manner diminishes image contrast. In the present study, we investigated the capabilities of both the raw (linear) OCT data and the logarithm of the OCT data for quantifying macrophage content within fibrous caps.

Before computing the image standard deviation, the OCT data within the ROI were preprocessed according to the following steps. First, the mean background noise level was subtracted, and then median filtering16 with a 3×3 square kernel was performed to remove speckle noise (IPLab Spectrum 3.1, Scanalytics). After preprocessing, σ within the ROI was calculated and tabulated for each specimen. To correct the data for variations in OCT system settings, σ was normalized by the maximum and minimum OCT signal present in the OCT image, as follows:

Figure 1. OCT and histology images of a fibroatheroma with superimposed ROIs. Raw (A) and base 10 logarithm (B) OCT images. C and D, Corresponding histology (C, Masson’s trichrome; D, CD68 immunoperoxidase; original magnification ×40).
where \( NSD \) is the normalized standard deviation of the OCT signal, \( S_{\text{max}} \) is the maximum OCT image value, and \( S_{\text{min}} \) is the minimum OCT image value.

The area percentage of CD68 and smooth muscle actin staining was quantified (at \( \times100 \) magnification) with automatic bimodal color segmentation within the corresponding ROIs of the digitized immunohistochemically stained slides (IPLab Spectrum 3.1, Scanalytics). The NSD within each cap was then compared with immunohistochemical staining from slides obtained from corresponding locations.

**Cap Thickness Measurement**
Both digitized histology and OCT measurements of cap thickness were taken at the center of each plaque (IPLab Spectrum 3.1, Scanalytics). A pathologist, blinded to the OCT results, morphometrically measured the fibrous cap thicknesses from digitized trichrome-stained slides. A total of 5 measurements by histology were obtained from each specimen. The maximum and minimum measurements were excluded, and the average of the remaining 3 measurements was used to comprise the data set.

**Statistics**
OCT measurements of macrophage and smooth muscle density were compared with histological measurements using linear regression. Because of the potential confounding association of OCT NSD with cap thickness, a partial correlation between the raw OCT NSD and CD68 percent staining, whereas for the base 10 logarithm OCT data, a correlation of \( r=0.47 \) (\( P<0.05 \)) was found between OCT NSD and CD68 percent staining. The partial correlation of raw OCT NSD and CD68 percent staining, controlling for cap thickness, was \( r=0.80 \) (\( P<0.0001 \)), indicating that OCT measurement of macrophage density is independent of cap thickness.

Morphometric evaluation of 26 slides stained with CD68 showed 9 caps with a CD68 area >10% and 17 caps with a CD68 area <10%. Receiver operating characteristic (ROC) curves for the raw and base 10 logarithm OCT signal NSDs are depicted in Figure 4. For the raw OCT signal NSD, a range of NSDs (6.15% to 6.35%) demonstrated 100% sensitivity and specificity (\( \kappa \) value 1.0) for differentiating caps containing >10% CD68 staining. For the base 10 logarithm OCT signal, NSD values ranging from 7.65% to 7.75% provided 70% sensitivity and 75% specificity (\( \kappa \) value 0.44) for identifying caps containing >10% CD68 staining. A comparison of the OCT NSD and CD68 staining is summarized in the Table.

**Smooth Muscle Cell Density**
A negative correlation was found between CD68 and smooth muscle actin percent area staining (\( r=-0.44, P<0.05 \)). In turn, a statistically significant negative relationship between smooth muscle cell density determined by immunohistochemistry and OCT NSD was observed for both the raw and base 10 logarithm OCT data. For the raw OCT data, a

\[
NSD = \frac{\sigma}{(S_{\text{max}} - S_{\text{min}})}
\]
correlation of $r = -0.56$ ($P < 0.005$) was found between OCT NSD and smooth muscle actin–positive percent staining, whereas for the base 10 logarithm OCT data, a correlation of $r = -0.32$ ($P = 0.12$) was found between OCT NSD and smooth muscle actin–positive percent staining. The partial correlation of the raw OCT NSD and smooth muscle actin percent area staining, controlling for cap thickness, was $r = -0.38$ ($P < 0.05$).

**Discussion**

Although many new approaches under investigation for plaque characterization show great promise, none provide direct evidence of macrophage presence. This study demonstrates that OCT is capable of visualizing macrophages and quantifying cap macrophage content. Because the OCT signal increases with the number of refractive index mismatches in tissue, caps containing macrophages should have multiple strong back reflections. A simple computational analysis of ROIs within OCT images of fibrous caps (NSD) was developed to test this hypothesis. When validated against immunohistochemistry, this parameter demonstrated a high degree of correlation with CD68 staining at corresponding locations ($r = 0.84$ for raw OCT data NSD).

Although little is known about the precise relationship between cap macrophage density and plaque vulnerability, studies have shown that plaques with a macrophage content in the range of 10% to 20% are more likely to be associated with unstable angina and non–Q-wave myocardial infarction. As a result, we selected 10% CD68 area as a cutoff for high macrophage content. Using the ROC to select an appropriate NSD threshold, we found that OCT was capable of accurately distinguishing fibrous caps with low macrophage content from fibrous caps with high macrophage content (100% sensitivity and specificity for raw OCT data NSD).

Studies have shown that macrophages are more abundant than smooth muscle cells in the plaques of patients with unstable angina. In the present study, we found an inverse correlation between CD68 and smooth muscle actin staining from corresponding locations within plaque caps ($r = -0.44$, $P < 0.05$). The negative correlation between the raw OCT data NSD and smooth muscle actin staining ($r = -0.56$, $P < 0.005$) may in part reflect the inverse relationship between macro-
phages and smooth muscle cells in our data. Nevertheless, it seems that the OCT NSD is specific for macrophage content, as opposed to being a more general metric of increased cellular density.

In the present study, both the raw OCT signal and the logarithm of the OCT signal were processed and compared with CD86 immunohistochemical positivity. Although the logarithm of the OCT signal provides an increased dynamic range for image display, it also apparently decreases the contrast between macrophages and surrounding matrix (Table). On the basis of our results here, we recommend that image quantification for determination of macrophage content be performed on the raw OCT signal.

**Alternative Methods for Identifying Inflammation in Atherosclerotic Plaques**

Diffuse near-infrared (NIR) reflectance spectroscopy is a quantitative approach that uses the spectrum of light scattered from within the vessel wall. A recent study using cadaver specimens has demonstrated that chemometric analysis of the NIR spectrum may allow identification of plaques containing abundant inflammatory cells. On the basis of the hypothesis that local inflammation within vulnerable plaques may lead to local elevations in temperature, studies have recently been performed using a temperature-sensing catheter. Experiments conducted in patients have indicated that both temperature heterogeneity and the temperature difference between atherosclerotic plaque and healthy vessel walls increase with disease severity. Both NIR spectroscopy and thermography seem promising for assessing inflammation within plaques, but these diagnostic techniques are not specific for macrophages and may need to be combined with another imaging modality to precisely determine whether the inflammatory cells are confined to the fibrous cap or are present throughout the plaque. Recently, ultrasmall superparamagnetic particles of iron oxide have been proposed for delineation of inflammatory changes accompanying atherosclerotic disease. The limited resolution of magnetic resonance imaging, however, renders the localization of macrophages within thin fibrous cap and plaque shoulders difficult.

**Study Limitations**

Because cadaver specimens were used in this study, 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 this study) on arterial optical properties is not known. However, a recent feasibility study, performed in patients, has shown that OCT images of coronary plaques obtained in vivo demonstrate similar features to those identified in this work (Figure 5). Therefore, it is likely that the OCT data analysis algorithms described here will also be applicable to intracoronary OCT images obtained from patients.

Because the resolution of the OCT system used in the present study was \( \approx 10 \mu m \), clear visualization of individual mononuclear macrophages was not possible. As a result, the NSD computed by the image-processing algorithm represents the reflectivity differences between collections of macrophages and surrounding cap matrix. Furthermore, sizes of macrophages vary widely, and the preprocessing step used to reduce speckle noise may remove information related to macrophage content. Future technology development to increase the resolution of OCT will reduce the dependence of quantitative macrophage determination on noise and could

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**Figure 4.** A and B, ROC curves for CD68 percent area cutoff of 10%. A, Raw data OCT NSD. B, Base 10-logarithm OCT NSD.

**Summary of Correlation Between Raw OCT Data and Logarithm OCT Data NSD vs CD68 Percent Staining**

<table>
<thead>
<tr>
<th></th>
<th>Raw OCT Signal</th>
<th>Logarithm OCT Signal</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Correlation, r</strong></td>
<td>0.84 (P&lt;0.0001)</td>
<td>0.47 (P&lt;0.05)</td>
</tr>
<tr>
<td><strong>NSD cutoff, %</strong></td>
<td>6.2</td>
<td>7.7</td>
</tr>
<tr>
<td><strong>Sensitivity</strong></td>
<td>1.0 (0.69 to 1.0)</td>
<td>0.70 (0.35 to 0.93)</td>
</tr>
<tr>
<td><strong>Specificity</strong></td>
<td>1.0 (0.8 to 1.0)</td>
<td>0.75 (0.48 to 0.93)</td>
</tr>
<tr>
<td><strong>Positive predictive value</strong></td>
<td>1.0 (0.69 to 1.0)</td>
<td>0.64 (0.3 to 0.89)</td>
</tr>
<tr>
<td><strong>Negative predictive value</strong></td>
<td>1.0 (0.8 to 1.0)</td>
<td>0.80 (0.52 to 0.96)</td>
</tr>
</tbody>
</table>

CD68 percent staining cutoff, 10%. Data in parenthesis represent 95% confidence intervals.
potentially enable the identification and tracking of individual macrophages within fibrous caps.

Selection of the location of macrophage density measurement in both OCT and histology may introduce a bias that could potentially affect the results of this study. To minimize this potential bias, we obtained measurements only at the center of each plaque. Registration of OCT and histology was accomplished by applying ink marks to the vessels at the imaging site. Because the outer diameter of the needle used to apply the ink marks was 450 μm (26 gauge), we estimate that the registration precision in this study was ≈500 μm. Because macrophage density may vary as a function of measurement location, our results may be affected by errors in registration between OCT images and corresponding histological sections. This limitation is present in all correlative studies of this type and must be considered when interpreting the results.

**Conclusion**

In conclusion, our results show that OCT is capable of accurately evaluating cap macrophage content. These findings are significant, because macrophage density is thought to be a major contributor in determining fibrous cap integrity. The ability of this imaging modality to quantify cap macrophage density is complementary to the high-resolution, cross-sectional visualization of plaque morphology provided by OCT. The simplicity of the image-processing algorithm used for macrophage evaluation should permit real-time superposition of this information during clinical imaging. These unique capabilities of OCT suggest that this technology may provide researchers and clinicians with a valuable tool for understanding the contributions of these inflammatory cells to plaque progression and rupture in patients.

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**References**


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