Identification of Lipid-Rich Plaques in Human Coronary Artery Autopsy Specimens by Near-Infrared Spectroscopy

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INTRODUCTION

A method is needed to identify non-stenotic, lipid-rich coronary plaques that are likely to cause acute coronary events. Near-Infrared spectroscopy (NIRS) offers simultaneous, non-destructive multicomponent analysis of biologic materials using optic energy in the NIR window of light. The NIR region includes wavelengths between 800 nm (near the red end of the visible spectrum) and 2500 nm (near the infrared region). Absorbance peaks in the NIR region originate from overtones and combinations of the fundamental bands and from electronic transitions in the heaviest atoms. For example, C-H, N-H, and O-H bonds are responsible for most major absorbances observed.

We have previously used NIRS to identify atherosclerotic plaque composition in human aortic and rabbit aortic plaques obtained at autopsy. This study was designed to test the hypothesis that NIRS can identify lipid-rich coronary plaques in human coronary specimens obtained at autopsy.

MATERIALS AND METHODS

Near-IR Equipment: A fiber optic probe (SmartProbe ™) of a FOSS/NIRSystems Model 6500 was used to measure the reflectance spectra over the spectral range 400 – 2500 nm (visible and near infrared). Coronary specimens were mounted on a 0.20 inch thick ceramic disk (5.0 in diameter) for scanning. An area of the ceramic disk was also used to record the reference baseline for the scan.
The probe has a circular window of approximately 7.9 mm that protects the fiber optic bundle. The probe has a central fiber bundle approximately 4.1 mm that brings the measuring beam from the monochromator. A concentric ring of 6.7 mm outside diameter receives the reflected light and returns it to the detectors in the instrument. (Figure 1).

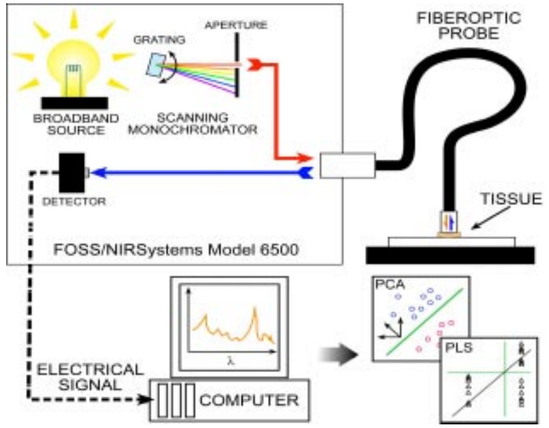

Figure 1. Near-InfraRed Spectroscopy System

Coronary spectra (Figure 2) were obtained in a darkened fume hood from 5 mm square coronary specimens (Figures 3-8). Bands from 400 to 650 nm are the bands of cytochromes. Bands due to the absorption of water are prominent at 1450 and 1934 nm. Bands between the large water peaks (at 1660 and 1830 nm) are primarily due to aliphatic and aromatic groups in the lipids and proteins of the tissue. Finally, absorption by the fiber optics themselves make the region beyond 2250 nm noisy and unusable.

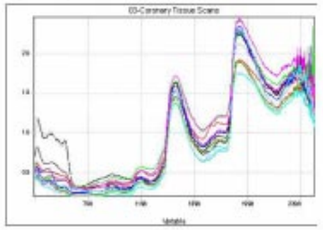

Figure 2. Near-infrared spectra from individual examples of human coronary tissue. Black= normal tissue; Green = atheroma; Blue= fibroatheroma; Purple= calcific plaque; Cyan fibrotic plaque, and Red= thin-cap fibroatheroma. As is clear from the spectra, there are no easily identifiable characteristics that permit determination of the composition of the sample. Such characterization requires furhter signal processing and chemometric analysis.

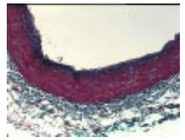

Figure 3. Normal Artery

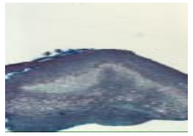

Figure 4. Atheroma

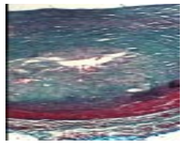

Figure 5. Fibroatheroma

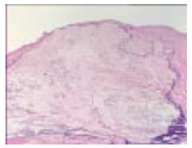

Figure 6. Calcific Plaque

Figure 7. Fibrotic Plaque

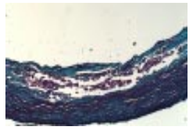

Figure 8. Vulnerable Plaque

Histology: A total of 45 human coronary arteries were obtained from 15 discarded cadaver hearts after forensic necropsy at the Medical Examiner Program in Frankfort, KY. A segment of each artery was sectioned at 5 mm intervals, yielding a total of 167 sections. None of the patients died from coronary heart disease. Hence, the plaques analyzed do not show the extremes of lipid content expected to be found in patients with acute coronary syndromes. After formalin fixation, all sections were subjected to initial spectroscopic data acquisition and subsequent histologic processing into paraffin blocks for embedment and tissue sectioning. A 5-micron microtome-generated section was cut from each paraffin block, mounted onto polylysine-coated glass slides, and stained with hematoxylin-eosin (H&E), and a combined elastic tissue- trichrome (ET) method. After processing, lipid appeared as predominately solvent-treated empty spaces in stained sections. We defined lipid pool as morphologically distinct spaces composed of clear, needle-shaped cholesterol clefts (representing ghosts outlines of dissolved crystals) and/or clear, bubbly, granular, mostly anucleate necrotic debris of foam cells. Taken all together, these are light microscopic characteristics of lipid gruel. Measurements were perrformed by light microscopy and computerized planimetry using the Zedex System Quantum Software. Twenty-one specimens were excluded because of sub-optimal histologic quality. Thus, a total of 146 coronary artery sections were used for analysis.

Data Analysis: Two chemometricians analyzed the data (DWH and BSW). NIRS data were divided into a training set (76 sections) and a validation set (70 sections). The training set was used to develop chemometric models to test the hypothesis in the validation set in a blinded fashion.

- ***Calibration within the training set:*** Before attempting predictions in the validation set, a calibration in the training set was constructed based on a subset of 12 samples determined by lipid area . This model was applied to the rest of the samples in the training set. Cross-validation, a method by which samples are removed one at a time from a model and then a prediction is attempted on the remaining samples, was used.
- ***Testing in the validation set:*** chemometric models of prediction were blindly applied to the validation set. The partial least squares- discriminative analysis (PLS-DA) was applied to identify presence or absence of lipid, and to measure lipid areas (mm2), employing the range 1680 to 1780 nm. Predictions were scored by a statistician (RK).

RESULTS

Histologic characteristics of the training and the validation sets are shown in Table 1. There were no significant differences between the groups.

Histology	Training Set	Validation Set
Normal	21 (27%)	19 (27%)
Atherosclerotic	55 (73%)	51 (73%)
Total	76 (100%)	70 (100%)
Intimal Thickening	15 (19%)	15 (21%)
Atheroma	8 (11%)	7 (10%)
Fibroatheroma	7 (9%)	6 (9%)
Calcific Plaque	4 (5%)	6 (9%)
Fibrotic Plaque	21 (28%)	17 (24%)
Plaque Area (mm2)	1.66 ± 1.21	1.43 ± 0.89
Lipid Area (mm2)	0.55 ± 0.57	0.40 ± 0.34

PLS-DA detected lipid areas ≥ 0.07 mm2 (presence of lipid) and < 0.07 mm2 (absence of lipid) with a 95% sensitivity and 96% specificity (Table 2).

	NIR (+)	NIR (-)	
Lipid (+)	21	1	Sensitivity: 95%
Lipid (-)	2	46	Specificity: 96%

Table 2. NIRS detection of lipid. All Samples in Validation Set (n=70), with averages when available. Results from the independent statistician. (PLS-DA model).

Models for NIRS predicted Vs. measured areas were developed for the training and the validation sets respectively as shown in Figure 8A and 8B.

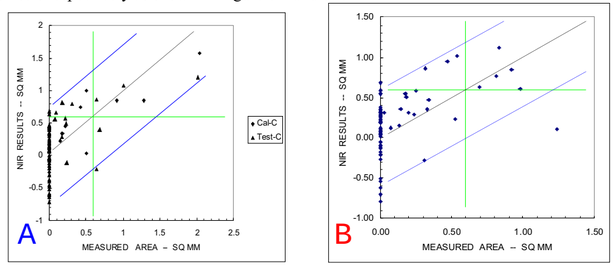

Figure 8. NIR prredicted (y Axis) Versus histology results (x axis). **A**=training set. **B**= validation set. Black lines are the perfect line of identity. Green lines drawn at 0.6 sq mm represent the values of lipid content that must be determined to be measured by NIR as different from zero at the 75% confidence level. Black diamonds in Figure A mark samples used in the initial calibration of 12 samples within the training set to determine the best calibration.

Results of the PLS model to detect lipid areas ≥ 0.6 mm2 (large lipid pool) and < 0.6 mm2 (small lipid pool) for the training and the validation sets are shown in tables 3 and 4 respectively.

TRAINING SET

	NIR (+)	NIR (-)	
Lipid (≥ 0.6 mm2)	6	2	Sensitivity: 75%
Lipid (< 0.6 mm2)	7	61	Specificity: 90%

Table 3. NIRS detection of lipid areas. All Samples in training set (n=76), with averages when available. Results from the independent statistician (PLS model).

VALIDATION SET

	NIR (+)	NIR (-)	
Lipid (≥ 0.6 mm2)	5	1	Sensitivity: 83%
Lipid (< 0.6 mm2)	4	60	Specificity: 94%

Table 4. NIRS detection of lipid areas. All samples in validation set (n=70), with averages when available. Results from the independent statistician (PLS model).

CONCLUSION

Near Infrared Spectroscopy can differentiate normal human coronary tissue from diseased atherosclerotic plaques. Furthermore, NIRS can classify coronary plaques according to their lipid content, the primary determinant of vulnerability. These findings support efforts to develop a Near-Infra-red catheter system to detect vulnerable coronary plaques in living patients.