Detection of Lipid-Rich Plaques With Near Infrared Spectroscopy In-Vivo

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INTRODUCTION

Lipid-rich vulnerable plaques that precipitate acute coronary events can not be detected by current diagnostic methods. We have previously demonstrated that near-infrared spectroscopy (NIRS) can detect lipid-rich plaques in autopsy specimens from atherosclerotic rabbits and humans. In the present study, we tested the hypothesis that NIRS can be performed with a laser-driven catheter system to identify lipid-rich plaques, through blood, in living rabbits with diet-induced atherosclerosis. The study included in-vivo NIRS identification of atherosclerotic and normal rabbit aorta, followed by discrimination between groups of lipid-rich versus lipid-poor atherosclerotic specimens.

MATERIALS AND METHODS

Near-IR Equipment: Modulated near-infrared laser light was delivered by an external-cavity diode laser into a 1.5 mm fiberoptic double-detector catheter (MedVenture, KY). The catheter focused the light in two opposite directions onto the vessel wall with a 45-degree wedge mirror located at the tip. Reflected photons were detected by a double-sided lead-sulfide sensor and converted into amplified electrical signals. The resultant signals were transmitted to the control computer for storage and analysis.
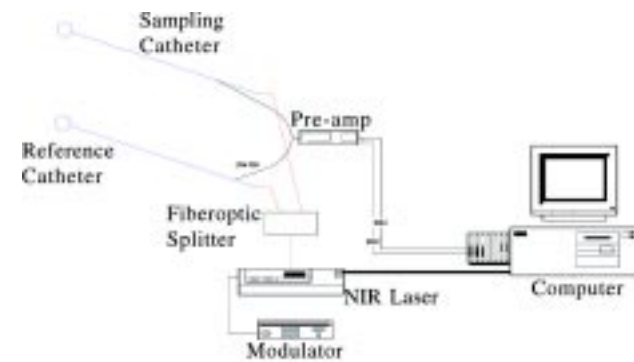

Figure 1. Near-IR Equipment consisting of near-infrared tunable laser, function generator, catheters, electrical amplifier, and computer.

Atherosclerosis Model: Twenty-three New Zealand rabbits on 1% cholesterol alternating with normal chow diet (8 months) and 4 normal rabbits were studied. Atherosclerotic plaques in this model are similar to those observed in humans (Fig 2). The study was approved by the committee on Research Animal Care of the University of Kentucky.
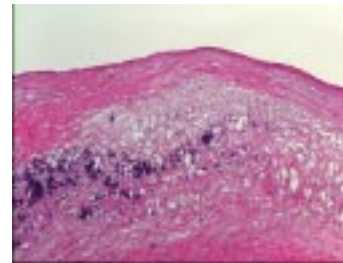

Fig 2. Rabbit Aortic Plaque

Experimental Protocol: After general anesthesia and intubation, a laparotomy was performed and the aorta exposed above the bifurcation. Full anticoagulation was achieved with IV heparin (100u/kg). NIRS retrograde catheterization was then performed using a 1.5 mm lead-sulfide, double-detector catheter (MedVenture, Louisville, KY). Each aorta was scanned with 2-nm resolution from 1720 to 1800 nm wavelengths. Spectral data collection was performed over a 15 cm length at 2.5-mm intervals for a total of 60 segments. Brisk blood flow was documented distal to the catheter in all cases. Silk marks were used for histologic colocalization analysis. After euthanasia, each aorta was fixed in 10% formalin and hemisected longitudinally into two equal circumferential (anterior and posterior) halves. The middle one-third (20) anterior segments were analyzed. Digital photographic recording of the intimal surface was followed by routine histology using the Hematoxylin and Eosin and the elastic trichrome staining. 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 performed by computerized planimetry using the Zedex System Quantum Software.

Data Analysis: To reduce noise contribution, collected spectra were averaged in data bins by lipid content determined by histology. Lipid content ranged from 0 to 1.9 mm^2, with a mean of 0.44 ± 0.03 mm^2. This provided twenty-two averaged scans for data analysis. Principal Component Analysis (PCA) was utilized to visualize the data and to determine feasibility for discrimination. Partial Least Squares (PLS) was implemented in the discrimination modeling to determine the capability of the system to differentiate between both the atherosclerotic and normal scans. PLS was also used to discriminate between lipid-rich and lipid-poor specimens. Cross-validation, a method by which samples are removed from a model one at a time from a model and then a prediction is attempted on those samples, was used to demonstrate the validity of the discrimination model.

RESULTS

Atherosclerotic Versus Normal Tissue: Analysis was performed to determine the capability of the NIR system to distinquish between atherosclerotic and normal rabbit aorta. PLS was used to form the discrimination model. Raw spectral data from the normal specimens were split into smaller bins averaged to give six total normal data points. As shown in Figure 3, the model demonstrated its prediction capability utilizing cross validation. Results are shown in Figure 4.
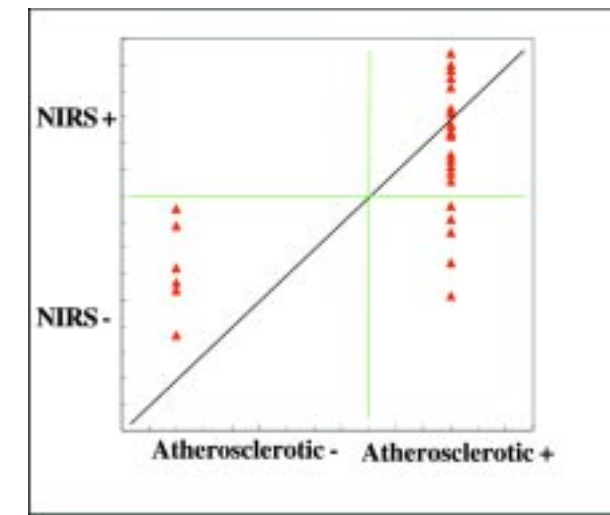

Figure 3. Plot of measured versus predicted lipid-pool areas in sq-mm. The horizontal green line identifies the discrimination value for the PLS model (below = normal, above = atherosclerotic)

	NIR +	NIR -	Total NIR	
Atherosclerotic +	19	5	24	79% Sensitivity
Atherosclerotic -	0	6	6	100% Specificity
Total Measured	19	11		

Figure 4. Near-Infrared spectroscopic prediction to identify atherosclerotic Versus normal tiisue. Cross validation using PLS.

Lipid-Rich Versus Lipid-Poor Plaques: This portion of the experiment focused on the technology's capabilities to distinguish between groups of lipid-rich Versus lipid-poor plaques. Principle component analysis was performed on eleven groups of plaques defined by lipid content (Figure 5).
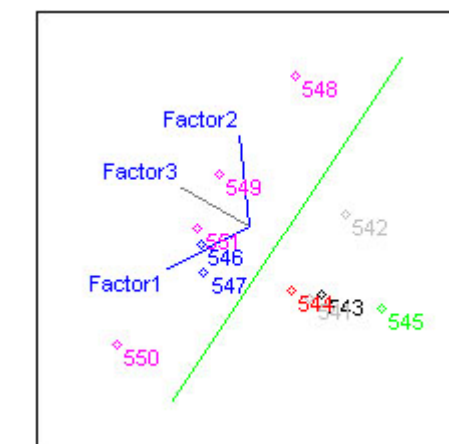

Figure 5. PCA plot of averaged scans. Points 541 - 545 contained ≤ 0.5mm^2, points 546-551 were from specimens containing > 0.5mm^2.

Chemometric modeling utilizing PLS for averaged scans of ≥ 0.75 and < 0.75 mm^2 (Figure 6).

	NIR +	NIR -	Total NIR	
Lipid-Rich +	7	2	9	78% Sensitivity
Lipid-Rich -	3	9	12	75% Specificity
Total Measured	10	11		

Figure 6. Near-Infrared spectroscopic prediction to identify groups of lipid-rich Versus lipid-poor plaques.

Conclusion: This intravascular, catheter based NIRS system was able to differentiate groups of lipid-rich from lipid-poor plaques, through blood, in the aortas of living rabbits. With further refinement of catheter design and reduction in noise levels, this system may be useful to identify individual lipid-rich plaques in the coronary arteries of living patients.