Laser Induced Spectroscopy of Cardiovascular Tissues

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Laser angioplasty is a new medical application of lasers that is being studied in an increasing number of laboratories worldwide. Laser radiation, delivered via flexible optical fiber, can remove fatty and fibrous deposits that build up inside the arteries and obstruct the normal flow of blood. Laser recanalization of vessels, which involves threading a small catheter percutaneously through the bloodstream to the occluded site, is an attractive alternative to invasive surgical procedures. A serious problem with this technique, however, is uncontrolled damage to the vessel wall, including perforation. Appropriate targeting of diseased plaque and monitoring of the ablation process can help avoid this type of catastrophic injury. Hence real-time diagnostic information about the diseased arterial site is virtually essential.

This work reports on laser induced tissue autofluorescence analysis which allows discrimination between diseased and healthy vessel wall, and which could aid in the safe application of ablative laser energy (1). The experiments so far have been conducted with cadaver tissues to establish the technique. Such spectral analysis will be particularly useful in laser angioplasty if similar results can be obtained under *in vivo* conditions.

Optical fiber guided argon ion laser radiation at 458 nm and XeF excimer laser radiation at 351 nm have been used to elicit autofluorescence spectra of fresh arterial wall samples in air and under blood. The autofluorescence profiles were detected by a spectrometer coupled to an optical multichannel analyzer. The experimental set-up is shown in Figure 1. Tissue sites were classified as either healthy arterial wall, lipid-rich tissue (early stage of atherosclerosis), or calcified plaque.

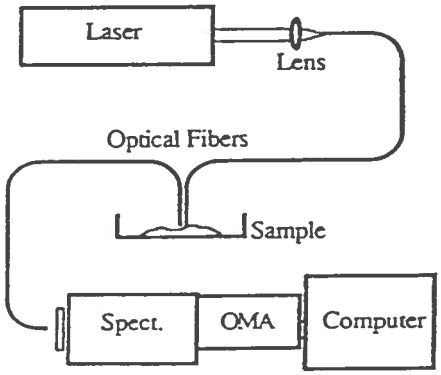

Figure 1. Experimental Set-up

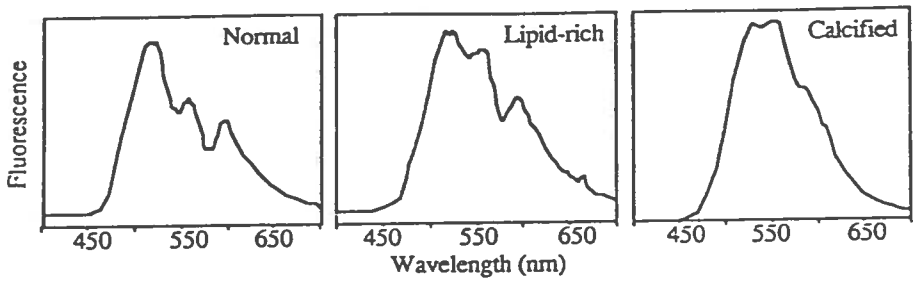

Figure 2. Argon Ion Laser Induced Arterial Wall Fluorescence

Typical 458 nm argon ion laser fluorescence profiles from all three tissue types are shown in Figure 2. Spectra excited at this wavelength from healthy wall exhibited three distinct peaks, at 520 nm, 555 nm, and 595 nm. This pattern of three discrete peaks was not seen at the diseased sites. In addition to this difference in spectral shape, the absolute fluorescence intensity was found to be much higher with atherosclerotic tissue. Changes in spectral shape and intensity corresponded to the degree of atherosclerosis, and made it possible to differentiate all three tissue types by fluorescence analysis.

Fluorescence profiles obtained by 351 nm excimer laser excitation are shown in Figure 3. The distinction between healthy and diseased tissue spectra is more subtle with this excitation wavelength. The disparity between the two profiles is more apparent in the difference curve in the lower portion of Figure 3. While healthy sites exhibit a consistent autofluorescence response, fatty or calcific sites produce a variety of spectral shapes. Careful subtraction analysis of spectra normalized to the same absolute intensity scale results in accurate histologic determination, as with the 458 nm fluorescence data. This is important because the excimer laser is an attractive candidate for angioplasty, due to its photoablative properties. Successful excimer-based diagnostics would allow for a clinical system requiring a single laser.

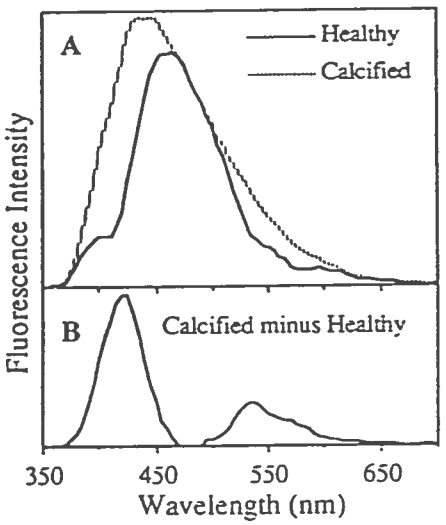

Figure 3. Excimer Laser Arterial Autofluorescence

The cause of the fluorescence response is open to debate. Most likely, many molecular agents within the arterial wall contribute in a complex process of photon emission and reabsorption which leads to the bulk tissue reaction observed. Flavins are a possible fluorescence source (2). Oxyhemoglobin, present in the wall, may play a reabsorptive roll in forming the "three peak" spectral shape seen in 458 nm induced profiles (3).

Using this fluorescence information, detailed images of the luminal surface of diseased arteries were made which clearly delineated regions of plaque (4). Sample were fixed flat with the endothelial surface exposed. Argon ion laser autofluorescence spectra were obtained at sites spaced every 2.5 mm along a two dimensional square grid. The maximum intensity in each spectrum at 520 nm was entered into a digital image processor and color-coded peak intensity maps of the luminal vessel surface were produced. These images clearly showed fine details in the distribution of the calcified tissue as well as grossly diseased areas. Presently, the obtained spatial resolution is approximately 1 mm. Using smaller diameter optical fiber it should be possible to reach 100 μm to 10 μm discrimination. This type of information could greatly aid in targeting laser ablation.

Spectral analysis can also be used to determine the appropriate endpoint of an ablation procedure, that is, when the diseased tissue layer has been completely removed and the healthy wall reached. Argon ion laser induced fluorescence spectra of a region of calcified plaque before and after excimer laser ablation are shown in Figure 4. An abrupt decrease in absolute intensity as well as change in profile shape are observed in samples when the calcified tissue has been completely penetrated (as confirmed by histologic analysis). Similar effects are seen with excimer laser analysis. These results agree with those obtained by mechanical removal of the calcified plaque. The 3 tissue types have been tested in this procedure, and peak intensity results are shown in Table 1.

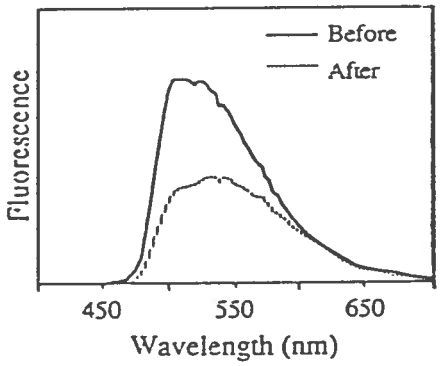

Figure 4. Autofluorescence at a Diseased Arterial Site before and after Ablative Plaque Removal

As presented in Table 1, the fluorescence intensity at normal sites rises with ablation into the tissue. It is possible that the different histologic layers of the arterial wall produce variations in the spectral response. This could prove advantageous for excimer laser-

based analysis. Because of the shallow penetration depth of ultraviolet light, a UV source could better discriminate thin layers of tissue by spectral investigation.

Table 1. Argon Ion Laser Autofluorescence Peak Intensity at Ablated Arterial Wall Sites

Arterial Tissue Type	Before Ablation	After Ablation
Healthy	2.9	5.2
Lipid-Rich	2.6	3.5
Calcified	23.5	6.5

Spectroscopy can also be used to directly monitor ablation. Excimer laser ablation of plaque in air produces a localized plasma plume containing ionic forms of calcium. Fluorescence profiles taken under these conditions will have sharp lines superimposed on the tissue response curve. These lines are only seen at calcified sites and only during ablative plume formation. This phenomenon could furnish very useful feedback information during the laser angioplasty procedure if it can be reproduced in a blood environment.

In summary, laser spectroscopy has been shown *in vitro* to provide information useful in directing photoablative recanalization. Autofluorescence analysis can be used to diagnose wall tissue as atherosclerotic and to assess the progress of plaque removal. This information should be important in implementing a safe laser angioplasty system.

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