INTRODUCTION

Atherosclerotic disease is a leading cause of death in the world today, and lesions of both coronary vessels and peripheral arteries are associated with increased morbidity and mortality (1). The occlusion of coronary vessels by atherosclerotic lesions typically results in ischemic heart disease. Considering its widespread prevalence, as well as its associated morbidity and mortality, effective treatment of atherosclerosis will be of great benefit to a vast number of patients. One example of a minimally traumatic intervention currently used to reduce atherosclerosis-induced stenosis is balloon dilatation of blood vessels (1). Unfortunately, despite the success of this technique, balloon angioplasty is not well suited for some complex lesion types, including total occlusion. Alternative minimally invasive modalities for reducing coronary atherosclerotic lesions should prove very important in the future as adjuvants or replacements to balloon angioplasty. One promising method is the use of high power laser light technology to ablate atherosclerotic lesions (2-5).

In the 1980s, various laser types were evaluated for their efficacy and safety for use in coronary angioplasty. Argon (514 nm) and Nd:YAG (neodymium:yttrium-aluminum-garnet) (1060 nm) continuous wave laser were two of the first to be evaluated clinically (6-8). These lasers destroy atherosclerotic lesions primarily via non-specific thermal action; in essence they burn the lesion. Unfortunately, both argon and Nd:YAG continuous wave induce considerable thermal damage to surrounding vessel tissue (9,10).

An alternative laser technology used for the ablation of obstructive coronary lesions is the excimer laser (10-13). Its active medium is composed of rare gas halides (binary halogens) that emit light in the ultra-violet...
The laser-light energy forces electrons from atoms, splitting chemical bonds. Thus the atherosclerotic lesion is destroyed in a photoablative rather than a thermal fashion. Fortunately, ultra-violet excimer lasers have proved more effective at clearing coronary lesions than other lasers evaluated, and are associated with little corollary thermal damage (10-13). Excimer laser removal of coronary atherosclerotic lesions has generated positive clinical and angiographic effects in patients. Notably, improvements have been recorded in patients with complex lesions (e.g., long, ostial, or total lesions) who receive excimer laser angioplasty as compared to traditional balloon angioplasty (14-23). Other studies, however, report no change, or even worse clinical outcome for patients receiving excimer laser versus balloon angioplasty for certain lesions (e.g., more than 10mm or in vessels with a reference diameter of more than 2.5mm) (24-26). As such, a more complete understanding of excimer laser performance is necessary to determine the clinical circumstances in which it will be most effective.

Despite the potential for improved patient outcomes with the use of excimer laser angioplasty, various report have indicated serious complications, most of which are related to the damage of neighboring, healthy vessel tissue. Notably, clinical trials and basic studies have uncovered the occurrence of tissue tears under the influence of shock waves (consisting of gaseous bubbles) that arise during laser illumination, which can lead to the development of serious complications during the postoperative period (10,22,27,28). It has been observed that local vessel saline infusion can reduce the severity of these shock waves, and thereby limit damage. In general, however, clinical outcomes following laser angioplasty would likely be improved and complications minimized by increasing the specificity of the laser action, thus limit the effects on healthy tissue, while at the same time maximizing its influence on the lesion.

In order to improve the therapeutic angioplasty and to minimize adverse effects, data concerning the reflection and absorption of energy, as well as the fluorescence of molecular structures within atherosclerotic plaques and normal tissue are essential (29-33). Spectral criteria for identification of vascular wall morphological types will help determine the optimal choice of laser energy and wavelength. Healthy, as well as primarily calcified, fibrous and fatty atherosclerotic lesions, considering their different molecular profiles, respond differently to laser light, and should not have exactly similar spectral characteristics. As such, healthy tissue and the various lesion types will have different wavelengths where an optimal ablative action is found. In general, by considering the reflection, absorption and fluorescence characteristics from healthy tissue and different lesion types, it will be possible to optimize conditions for lesion ablation and to minimize laser action and thus damage to normal vessel tissue.

In order to achieve this specificity, answers to two principal questions must be found. First, to attain the greatest lesion ablation, it is critical to determine in what region of the spectrum (specific wavelengths) atherosclerotic plaques exhibit maximal adsorption of laser light. Moreover, to minimize damage to healthy vessel tissue, it is necessary to determine the minimal adsorption wavelengths of the intact, healthy vessel. One way to approach this is a detailed experimental study of the spectral properties of atherosclerotic plaques and normal vessel wall tissue. Secondly, it must be determined whether there are optical characteristics of atherosclerotic plaques and of normal tissue that will permit them to be reliably distinguished. This would make it possible to establish preconditions for identification of a particular type of atherosclerotic lesion.

The present work has endeavored to define and evaluate several spectroscopic parameters of atherosclerotic and normal human aortic wall. To achieve this goal, the following tasks were undertaken: (i) to define the spectral parameters of healthy and atherosclerotic aortic wall; (ii) to compare the spectral characteristics of various types of atherosclerotic plaques with the spectral characteristics of the normal aortic wall; and (iii) to compare aortic wall morphology with data from spectral analysis. It is anticipated that this data will provide information that will be useful in the prediction of an optimal energy of laser for angioplasty and diagnosis of different atherosclerotic plaques morphological types. It must, however, be stressed that these data will need to be confirmed both in vivo and in coronary arteries before any definite clinical predictions can be made.

**METHODS**

The present work was conducted on 25 fragments of aortic arch from persons of both sexes (aged 50 to 80 years) who died of various causes. The experimental group consisted of samples of atherosclerotic aorta (lipidic, fibrotic and calcified plaques), while samples of normal aortic wall served as controls.

**Generation of the Absorbance Spectrum**

Cryostat sections of normal and lesioned aortic samples were used to record spectral absorbance in the ultraviolet range. Sections of 10 to 15µm thickness were placed between two pieces of quartz glass. Absorbance spectra were recorded with a SP-26 scanning spectrometer, which measures absorbance spectrum in...
the range of 180 to 1100nm. A DVK-3 computer increased the sensitivity of the apparatus and enabled automated processing of the spectrophotometric signal with construction of a spectral absorption curve. The absorbance spectra were plotted as curves whose peaks correspond to the optical density of the substance.

**Recording of Reflection Spectra**

For recording of reflection spectra, preparations of atherosclerotic and normal aortic wall were used. The specimens were placed on a stand at an angle of 45° to the direction of the beam of incident light. The excitation source was a Specol-10 with halogen lamp (retuning range 300 - 800nm); spectra were recorded with the aid of a spectrophotometer on a monochromator base with a diffraction grating (MDG-23) of resolution 6Å/mm. Light was focused onto the specimen to a section of 0.5 x 0.5cm through a micro-objective. With the aid of an achromatic condenser (focal distance 90mm), reflected light was directed onto the entry slit of the MDG-23 monochromator; the light was subsequently directed through the exit slit onto a photoelectron multiplier (PhEM-39). The signal from the PhEM was processed by an analog-numerical transformer and the result was depicted graphically on the display screen of the DVK-3 computer. The graph depicted the dependence of intensity of reflected light (number of photons per second) on wavelength (nm). The instrument error was 10%. The program for processing the results of measurements allowed time to accumulate readings from the instrument (photon counter) and also to conduct smoothing, scaling and elimination of incidental erroneous spikes.

**Recording of Fluorescence Spectra**

Cryostat sections of normal and atherosclerotic portions of aortic wall were used for study of fluorescence spectra. Specimens were prepared within 12 hours of death by layered adhesion of acetone-fixed cryostatic sections to glass. These samples were generally 120 to 130µm thick, with an area of 10 x 15mm. The samples were fixed on a special object platform at an angle of 45° to the direction of the beam.

![Figure 1A](image1.png)

Figure 1A. The absorption spectra of normal aorta wall (solid line) and atherosclerotic plaque (dashed line) upon exposure to laser light in the ultraviolet range.

![Figure 1B](image2.png)

Figure 1B. Absorbance of normal aorta wall (black) and atherosclerotic plaque (grey) at 280 nm. * p < 0.05, Wilcoxon test.
of incident light. The sample was excited by the lines of a DRSh-500 mercury lamp (with maximal excitation in the range of 313 and 365nm), isolated with the aid of a MUM-1 monochromator with a slit spectral width of 1nm. Sample luminescence was detected by an SPSh-4 prism monochromator with a slit spectral width of 3nm and transformed in a photoelectron multiplier (PhEU-79), after which the signal entered a constant current amplifier and was registered on a chart recorder (KSP-4) as a graph of the fluorescence spectrum. The graph depicted the dependence of the fluorescence intensity of the sample (standard units) on wavelength (nm). The relative error of the prism monochromator with photoelectric recording was 5 to 10%.

RESULTS

Absorbance Spectra of Atherosclerotic and Normal Aortic Tissue

Upon comparison of absorbance spectra from normal aortic tissue and from atherosclerotic plaque (without differentiation according to plaque type), two characteristic optical density maximums are observed at wavelengths 224 and 280nm. At the first maximum, the difference between the indices of optical density were not significant. Consequently, further analysis of light absorbance by aortic wall tissue at this wavelength was not conducted. In the first curve (Figure 1A), which corresponds to the absorbance spectrum for normal aortic wall in the ultraviolet range, the slope decreases upon approaching the second maximum, which corresponds to a wavelength of 280nm. This is of greatest interest. At this point, the optical density of normal tissue is 0.65 ± 0.02 standard units. The second curve, which corresponds to the absorbance spectrum of atherosclerotic tissue, declines more steeply and forms a maximum at the same wavelength with an optical density of 0.46 ± 0.02 standard units. The upward and downward slopes are approximately identical in the two absorption graphs: in the first, the absorption spectral curve declines to an optical density of 0.12 ± 0.004 standard units; in the second, to 0.03 ± 0.00035 standard units. Thus, in the ultraviolet (280nm) absorption spectra of normal and atherosclerotic tissues, there is a statistically significant ($p < 0.05$; Wilcoxon test), 30% decrease in the optical density of plaque tissue in comparison to intact tissue from aortic wall (Figure 1B).

Reflection Spectra of Atherosclerotic and Normal Aortic Tissue

In reflection spectra, it is apparent that the 520 to 770nm visible wavelength range is a promising region for the exploitation of laser energy for surgical purposes (Figure 2A). Here, the difference in the reflection spectral behavior of atherosclerotic plaques and normal tissue is quite strong. The following characteristic peaks are found in the reflection spectra within the visible range.
range. The first peak corresponds to a wavelength of 625nm. Here, tissue from normal aortic wall reflects, on average, 92.5 ± 2.5% of incident light; samples with lipidic and fibrotic plaques reflect 62.5 ± 2.5%; in comparison, the coefficient of reflection for samples with calcified plaques is 39 ± 1.0%. The next reflection peak corresponds to 565nm. Here, normal tissue from aortic wall reflects, on average, 59.0 ± 1.0% of incident light; lipidic and fibrotic plaques, taken as a single group, reflected 31.5 ± 1.5%; and samples with calcified plaques reflected 16.0 ± 1.0% of incident light. The last peak lies in the vicinity of 525nm. As before, a pronounced reflection coefficient of 55.0 ± 0.5% was observed for normal tissue; samples with lipidic and fibrotic plaques, considered as one group, reflect on average 36.0 ± 1.0% of incident light; calcified plaques exhibit reflectivity of negligible intensity, namely, 24.0 ± 1.0% of incident light. All differences between mean reflectivity indices of these groups are statistically reliable (p < 0.05) (Wilkoxon test). Thus, at all points of the visible spectrum studied, the reflectivity of normal aortic wall tissue samples is significantly greater than the analogous measurement for samples with visually defined atherosclerotic lesions (Figure 2B).

**Fluorescence Spectra of Atherosclerotic and Normal Aortic Tissue**

The fluorescence spectrum of normal tissue is represented by a symmetric curve with a maximum fluorescence in the vicinity of 470 ± 5nm. The excitation maximum in this instance lies near 365nm (Figure 3A). For lipidic and fibrotic plaques, the fluorescence intensity is significantly greater than for normal tissue and the fluorescence maximum is shifted to the shorter wavelength of 440 ± 5nm. The spectrum is represented by a symmetric, broadened band, the appearance of which depends on the degree of tissue damage. The fluorescence spectrum of the calcified plaque has the appearance of an asymmetric curve with two fluorescence maxima in the vicinity of 570 and 380nm. The latter maximum can be better distinguished by illuminating tissue with light of 313nm wavelength (excitatory light).

To quantitate differences between fluorescence spectra, the ratios of fluorescence intensities for normal or atherosclerotic aortic wall tissue at optimal wavelengths (440 to 470nm and 500 to 570nm) were measured at a single excitation wavelength (Figure 3B). For normal tissue, the 440 to 470nm ratio was 0.843 ± 0.042, while the 500 to 570nm ratio was 0.29 ± 0.051. At the same ratios, the corresponding indices for fibrotic and lipidic plaques were 1.19 ± 0.035 and 0.56 ± 0.012, respectively. Calcified plaques were characterized by the following indices: the 440 to 470nm ratio was 0.39 ± 0.035 and the 500 to 570nm ratio was 0.42 ± 0.021. All observed differences were statistically reliable to p < 0.05 (Wilkoxon test) (Figure 3B).
DISCUSSION

The absorbance, reflection and fluorescence spectra of normal and atherosclerotic aortic wall tissue were defined. The data indicate that the visible range (principally red; 520 - 770nm) should be investigated further for use in laser angioplasty. In this region, on the basis of reflection and fluorescence spectra, the greatest optical differences are exhibited between intact and atherosclerotic vessel wall. The findings of these experiments are generally in agreement with other groups’ findings suggesting that it is possible to distinguish between atherosclerotic lesions and healthy vessel tissue (29-33).

The manifestation of spectral differences between normal and atherosclerotic aortic wall is dependent upon: (i) infiltration of vessel wall by lipids, cholesterol and its ethers, which lower the optical density of the plaque and lead to a lowering of the absorption capacity of the tissue; (ii) the density of the tissue under examination, such that the higher the density (calcinosis), less light is reflected and, conversely, the lower the density (normal tissue), more light is reflected; and (iii) the degree of infiltration of the vessel wall by substances possessing a high capacity for luminescence (lipids) (34).

The data obtained in the current experiments are consistent with these attributes. Upon comparison of the spectral characteristics of various types of atherosclerotic plaques and normal aortic wall tissue, statistically reliable distinctions were exhibited in the following spectra. In absorption spectra in the ultraviolet range (280 nm), there is an average lowering of 30% in the optical density of plaque tissue, in comparison to intact aortic wall tissue. In reflection spectra the intensity of reflection for samples with lipidic and fibrotic plaques was 30 - 50% lower than normal aortic wall tissue at all points of the visible spectrum that were examined (625, 565 and 525 nm); calcified plaques were 60 - 75% lower. In fluorescence spectra the ratio of fluorescence intensity at wavelengths 440 and 470nm (J440/470) may serve as a diagnostic parameter. If J440/470 slightly less than one, this is an indicator of normal aortic wall tissue; however, if J440/470 greater than one, it likely indicates the presence of fibrotic and lipidic plaques. In the event that J440/470 is equal to one, the possibility of transition from normal tissue to plaque must be considered. Finally, a J440/470 much less than one is likely to represent a calcified plaque.

The spectral characteristics obtained in these experiments are interpreted in terms of the basic stages of atherosclerosis, which have variable molecular composition (i.e., the relative levels of different molecules such as lipids and mucopolysaccharides). In this regard, it may be proposed that lipids and mucopolysaccharides, infiltrating the vessel wall in the stage of lipidosis and liposclerosis, lower the optical density of the tissue and absorb light less than normal (see above). This was the cause of the characteristic changes observed in absorption spectra recorded from normal aortic wall tissue and atherosclerotic tissue (lipidic and fibrotic plaques) (Figure 1).

The levels of light reflection from the surfaces of normal and atherosclerotic aortic wall differ. Moreover, the higher the density of the tissue studied, the less light it reflects (see above). This conclusion is supported by the recorded reflection spectra: normal aortic tissue has the maximal reflection intensity, while the densest medium (calcified plaque) absorbs the major share of incident light (Figure 2).

Results obtained with the aid of such precise and informative methods as the recording of fluorescence spectra also indicate differences. Indeed, the greater the tissue content of lipid, cholesterol and its ethers, the more powerfully it will luminesce, inasmuch as these substances, according to published data, possess this property to a marked degree (see above). Keeping the foregoing in mind, one may predict that lipidic and fibrotic plaques will possess the greatest fluorescence index, while normal tissue will fluoresce to a lesser degree and calcified plaques will luminesce quite weakly. This is supported by the experimental recordings of fluorescence spectra (Figure 3).

There are various limitations of the current study that should be mentioned. All the measurements made were in cryostat sections of post-mortem cadaverous aorta. As such, before more definite claims to clinical relevance can be made, it will be necessary to confirm these findings using an in vivo model. Nevertheless, in vitro measurements of thermal laser induced damage have proved to be good predictors of in vivo damage (9). Another potential problem is that the detection of subtle features such as slopes of the spectral curves or changes in fluorescence intensity may not be reliable in practice in the clinical setting. However, improvement in laser technology will likely make these issues less of a concern. A final potential limitation is that the measurements were taken from atherosclerotic and normal aortic tissue. As laser angioplasty is typically envisioned for ablation of coronary artery lesions, it will be important to confirm these data in these tissues.

The ultimate goal of this and other studies is to find a simple and safe operational system for the diagnosis of plaque type and the definition of the boundaries of the atherosclerotic lesion; these measurements are of the greatest importance for direction and delivery of intravascular laser energy. Ideally, clinical laser angioplasty should be conducted with a scanning
protocol (to determine the plaque type and to recognize healthy vessel wall), permitting the laser wavelength to be periodically corrected with respect to spectral properties and the relief of the illuminated plaque surface. This should optimize ablation and minimize damage to healthy vessel tissue by allowing adjustment of laser energy according to lesion type.

In summary, this work has evaluated various spectral characteristics of atherosclerotic vs. normal aortic tissue and finds wavelengths where greater differences exist between atherosclerotic and normal tissue. Thus, these wavelengths should be further investigated. Despite the limitations discussed above, the current set of experiments provide the groundwork for a more complete understanding of laser-tissue interactions that may eventually result in an improvement of clinical technique.

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