Excimer Laser–Induced Simultaneous Ablation and Spectral Identification of Normal and Atherosclerotic Arterial Tissue Layers

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A krypton-fluorine excimer laser at a 248-nm wavelength was used to irradiate normal and severely atherosclerotic segments of human postmortem femoral arteries. Single pulses and multiple pulses required for penetration or perforation of the arterial wall were applied with 16 nsec pulse width and 5 J/cm²/pulse energy fluence. The total fluorescence of irradiated and ablated tissue was analyzed in real-time mode by means of spectroscopy. Each laser pulse produced one spectrum that was characteristic of the composition of the tissue layer, which was ablated. Fluorescence spectroscopy indicated a broad-continuum emission between 300 and 700 nm with peak fluorescence of equal intensity at wavelengths of 370 and 460 nm (ratio, 1.004 ± 0.087) for normal media layers. Atheromas without calcification (lipid, fibrous, and mixed) were found with spectral maxima at the same wavelengths but with significantly reduced intensity at 460 nm (ratio, 1.765 ± 0.263; p<0.001). In contrast to this broad-continuum fluorescence, calcified plaques displayed multiple-line emission with the most prominent peaks at wavelengths of 397, 442, 450, 461, 528, and 558 nm. These fluorescence criteria identified the histologically classified target tissue precisely. Histological examination of the corresponding arterial layers indicated sharply delineated and circumscribed tissue ablation. These results indicate that simultaneous tissue identification (diagnosis) and ablation (treatment) by excimer laser irradiation is feasible under strict laboratory conditions. We conclude that this principle demonstrates the potential for laser beam control by means of target-specific ablation. (Circulation 1988;78:1031-1039)

Laser beams have been used in different fields of medicine as an instrument for cutting and coagulation,1–3 and their potential to remove atherosclerotic artery obstructions has been investigated in vitro and in vivo. Clinical trials have used either operative4,5 or percutaneous6–9 approaches. However, the lack of control over the laser beam limits the safety and, therefore, clinical efficiency of this technique. Laser systems most commonly applied in these clinical settings have been Nd: YAG-, argon-, and carbon dioxide–lasers, operating either with continuous waves or with long pulse durations. Their cutting ability depends upon the transfer of laser beam energy into heat in the tissue target until thermic vaporization commences.10,11 Tissue ablation induced by this mechanism is associated with charring and coagulation necrosis of vascular tissue layers around the laser target, and laser ablation may produce thrombosis, vasospasm, aneurysm formation, and even perforation.

Excimer lasers are pulsed ultraviolet lasers and are under intensive investigation for their potential application in the treatment of cardiovascular disease. In vitro, they have produced precise ablation of vascular tissue without injury to the surrounding structures. The ablative (cutting) mechanism of excimer lasers is generally thought to rely on the phenomenon of “ablative photodecomposition” described initially by Srinivasan and Mayne-Banton in 1982.12 However, like other laser beams, this laser pulse train cannot differentiate between normal and atherosclerotic tissue layers.

When excimer laser irradiation interacts with biological tissue, grossly detectable fluorescence is emitted from the laser target. This study evaluated the excimer laser–induced simultaneous tissue abla-
tion and fluorescence of different human arterial tissue layers from the aspects of real-time tissue characterization and differentiation. Ablation and fluorescence patterns were investigated to determine whether fluorescence analysis might be useful to control the action of subsequent laser pulses by combining the therapeutic and diagnostic potential of excimer lasers.

Materials and Methods

Laser

The experimental setup is shown in Figure 1. Laser energy was supplied by an excimer (Excited Dimer) laser (Technolas MAX-10, Munich, FRG). The krypton-fluorine wavelength of 248 nm and a pulse length of 16 nsec were used. The laser emitted up to 400 mJ/pulse of laser energy, which was monitored by an energy meter and controlled by a microprocessor that maintained the high voltage in such a way that the laser output was kept constant during the experiment. The native laser beam was focused by an optical lens with a focus length of 30 cm. The laser power was adjusted so that an energy fluence of 5 J/cm²/pulse was obtained in the target of the laser beam. Energy fluence was therefore in a range well above the ablation threshold for biological tissue and was high enough to produce the effect of "ablative photodecomposition." All experiments were performed at repetition rates of 5 Hz (5 pulses/sec). No higher repetition rates were applied because the analyzing unit in the setup was not able to process the data input faster. Tissue samples were placed at such a distance that a laser spot diameter of 2 mm was achieved. Air was used as a laser-tissue interface. An air stream was not applied to the target to remove degradation products.

Fluorescence Analysis

Fluorescence emitted from the irradiated target was guided by a 1-mm diameter quartz core fiber, 150 cm in length. The fiber was placed 10 cm away from the laser target at an angle of 45° (according to the laser beam direction). An ultraviolet-blocking filter was placed in front of the fiber tip to exclude all wavelengths less than 300 nm from the analysis and to partially exclude those between 300 and 350 nm. This setup eliminated all the laser light reflected from the tissue target, and it protected the diode array. The fiber was coupled to a spectrograph and O-SMA optical simultaneous multichannel spectral analyzer system (Spectroscopy Instruments, Munich, FRG). Tissue fluorescence was spectrally dispersed by a 27-cm spectrograph (PTI-200, diffraction grating 150 l/mm) and covered a region of 200–1,000 nm. The wavelength resolution was approximately 1 nm. The spectral pattern was imaged onto an optical multichannel analyzer detector head (IRY-1024, Princeton Instruments, Princeton, New Jersey), consisting of a linear diode array of 1,024 elements. Diode signal outputs were gathered by a detector controller (ST-120, Spectroscopy Instruments) and were further processed, collected, and stored by a computer (IBM-AT) with appropriate software. All analyses were carried out in real-time mode. Fluorescence-emission spectra were displayed on a computer screen and traced by a plotter. Background light was subtracted from each fluorescence spectrum for the purpose of eliminating noise signals from different sources.

The O-SMA was triggered to the onset of laser beam delivery, and this allowed the imaging of tissue fluorescence during 16 nsec of laser irradiation and the following 3,500 nsec. Because excimer laser–excited tissue fluorescence lasts no longer than 3,400 nsec (unpublished results from our laboratory), the total fluorescence induced by one excimer laser pulse was recorded and analyzed. In this way, one laser pulse produced one spectrum, and a sequence of laser pulses resulted in a sequence of spectra that were displayed in a three-dimensional, real-time manner on the computer screen. Calibration was performed and checked repeatedly with the spectral emission of a mercury and a neon vapor lamp. Fluorescence intensity was measured in arbitrary units (relative intensity).

Tissue Samples

Six- to twelve-hour postmortem human arterial segments from common or superficial femoral arteries were used as laser targets. Fourteen samples without any evidence of atherosclerotic disease, which was confirmed by retrospective (after laser) light microscopy, were taken from five individuals between 6 and 48 years of age. Sixteen segments with different types and severity of atheromas were removed from four individuals between 44 and 83 years of age. The pathological tissue lesions involved atheromatous plaques of lipid (yellow plaque), fibrous (white plaque), mixed (lipid and fibrous plaques), and calcified types and were considered to represent the variety of chemical and physical structures in human atherosclerosis. Atheromas associ-
ated with gross evidence of thrombi were not used for this investigation. If microthrombi were found during light microscopy, the tissue and corresponding spectra were excluded from the study. The arteries were split longitudinally and were exposed to the perpendicularly arranged laser beam. Both normal and pathological samples were irradiated at the luminal (intima) or outer (adventitia) surface. Experiments were performed either as single-pulse or multiple-pulse studies. Laser craters resulting from multiple-pulse exposure of atherosclerotic lesions were classified histologically as penetration, advanced penetration, or perforation according to ablation depth. Plaque ablation that penetrated the underlying medial wall layers was classified as advanced penetration ("imminent perforation"). Laser craters where the ablation either affected the adventitial layer or removed that layer completely were classified as perforations (wall perforation). Exposure of multiple-pulse studies was modified from 10 to 60 seconds by steps of 5 seconds, and exposure was concluded earlier if grossly detectable wall perforation occurred. All arterial samples were irrigated in saline solution at the time of collection and immediately before laser exposure to remove erythrocytes and hemoglobin deposits that might interfere with native arterial spectra.

After laser irradiation, femoral artery segments were inspected for laser ablation–associated injuries of surrounding structures and penetration depth. Tissues were subsequently fixed in formalin for histological examination. They were embedded in paraffin, and they were sectioned and stained with hematoxylin and eosin, van Gieson’s stain, and Gomori’s trichrom stain. Tissue layers identified by microscopy and that were penetrated by the excimer laser beam were compared with and related to the corresponding fluorescence spectrum. Morphometry was carried out for assessment of penetration depth and calculation of ablation rate.

Statistical Methods

Values obtained from spectral analysis were expressed as relative fluorescence intensities (arbitrary units) at each wavelength. For statistical comparison, the ratio of the most prominent fluorescence intensities at wavelengths of 370 and 460 nm was derived, and mean±SD values of this ratio were calculated for the following, histologically confirmed groups of tissue: normal media, adventitia, lipid, fibrous, and mixed plaques. Because of the high ablation rate and the generally very thin normal intima, no spectra were recorded that contained information about that particular layer alone. Analysis of variance and the multiple comparison Student-Newman-Keuls test were used to compare the 370 nm:460 nm fluorescence intensity ratios of different tissue layers. Statistical significance was assumed as p<0.01.

Results

Normal Media

A typical fluorescence spectrum for normal media wall layers of a common femoral artery is shown as the original record in Figure 2. This spectrum was characterized by a broadband emission between 300 and 700 nm and displayed a maximal fluorescence intensity at wavelengths of 370 and 460 nm. Both maxima were of equal relative intensity with a 370 nm:460 nm intensity ratio of 1.004±0.087 from the 25 spectra analyzed and with confirmed histology of this tissue type (Table 1). Spectroscopic discrimination of adventitia and atherosclerotic lesions from the normal media by the 370 nm:460 nm ratio was highly significant (p<0.001).
Adventitia

Although all fluorescence spectra from normal media were characterized by an “equal intensity double peak” feature, the adventitia exhibited a different fluorescence intensity ratio. This tissue type released broadband emission with peaks at the same wavelengths (370 and 460 nm) and similar spectral morphology but higher relative intensity at 460 nm, expressed as the mean ratio 0.812 ± 0.060 (Figure 3, Table 1). The ratio revealed significant (p<0.001) differences from all other tissue layers analyzed. Results from fluorescence analysis for media and adventitia were identical, regardless of whether the vessel wall was irradiated or penetrated from the inside to the outside or vice versa.

Noncalcified Atheroma

In contrast to wall layers without histological evidence of atherosclerosis, atheromas consisting predominantly of either lipid or fibrous material or both resulted in a fluorescence pattern similar to that depicted in Figure 4. A broadband emission from the laser target was again evident throughout a wavelength range of 300 to 700 nm with the same maxima at 370 and 460 nm, respectively, but the long wavelength peak in this case was significantly (p<0.001, Table 1) lower than that at the short wavelength compared with spectra from other layers. The 370 nm:460 nm fluorescence intensity ratio was 1.765 ± 0.263 in 71 spectra obtained and analyzed from noncalcified femoral lesions. Classification of atheroma morphology in lipid, fibrous, and mixed pathology by the intensity ratio revealed a lack of significance (p>0.05, Table 1). Presence of slight lipid deposits in artery segments, which had been classified as “normal” by gross examination, was detected first by the characteristic fluorescence pattern and confirmed subsequently by histological studies.

Calcified Atheroma

Figure 5 displays the fluorescence spectrum obtained during ablation of an atheroma with calcium deposits. The spectral morphology is characterized by a multiple, strong-line emission throughout the visible wavelengths. On the basis of fluorescence analysis, the distinction between tissue layers with and without calcium is quite strong, for the differences are not only found in the quan-

TABLE 1. Spectral Characteristics of Different Arterial Tissue

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Analyzed spectra (n)</th>
<th>Spectral morphology</th>
<th>Peak emission (nm)</th>
<th>Relative intensity 370:460 nm ratio (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Media</td>
<td>25</td>
<td>Double peak</td>
<td>370, 460</td>
<td>1.004 ± 0.087</td>
</tr>
<tr>
<td>Adventitia</td>
<td>20</td>
<td>Double peak</td>
<td>370, 460</td>
<td>0.812 ± 0.060</td>
</tr>
<tr>
<td>Lipid plaque</td>
<td>27</td>
<td>Double peak</td>
<td>370, 460</td>
<td>1.706 ± 0.262</td>
</tr>
<tr>
<td>Fibrous plaque</td>
<td>25</td>
<td>Double peak</td>
<td>370, 460</td>
<td>1.805 ± 0.266</td>
</tr>
<tr>
<td>Mixed plaque</td>
<td>19</td>
<td>Double peak</td>
<td>370, 460</td>
<td>1.794 ± 0.259</td>
</tr>
<tr>
<td>Calcified plaque</td>
<td>15</td>
<td>Multiple peak</td>
<td>397, 442, 450</td>
<td>No ratio calculated</td>
</tr>
</tbody>
</table>

![Figure 3. Fluorescence spectrum of adventitia displays broadband emission throughout the same spectral range as the media with decreased 370 nm:460 nm ratio (0.812±0.060). Superficial femoral artery, 35-year-old man.](image-url)
titative but are more significant in the qualitative distribution of emission maxima. The most important peak emission with more than 40% relative fluorescence intensity was found at wavelengths of 397, 442, 450, 461, 528, and 558 nm (Figure 5).

Three-dimensional Spectral Profile of Atherosclerotic Plaque Ablation

Results were derived from 18 advanced penetrations and 12 perforations. Three-dimensional spectral recordings of multiple-pulse laser exposures with associated advanced penetration or perforation of the arterial wall displayed the spectral profile of subsequently irradiated and ablated arterial tissue layers (Figure 6). Type of atherosclerotic lesion (noncalcified and calcified), underlying attenuated media, and adventitia were correctly identified and correlated with the depth of penetration as assessed by histological examination and microscopic layer morphology. The event of wall perforation was preceded by at least 10 (19.4±8.2) media-type fluorescence spectra, which indicated that the laser pulses had completely ablated the atherosclerotic layers and had entered the thinned underlying normal arterial wall. The number of spectra suggestive of medial layers before perforation occurred is shown in Table 2. Therefore, spectral analysis detected medial layers in 12 perforations before the perforations actually occurred and in 16 of 18 advanced penetrations.

Impact of Ultraviolet Filter

Results in this setup were influenced by the ultraviolet filter. The filter was capable of blocking emission in the ultraviolet spectral range up to a wavelength of 350 nm. The uniform fluorescence pattern of
all tissue layers in the displayed range of 300 to 350 nm (constant decrease of relative intensity at lower wavelengths) must be explained as a result of selective absorption by the filter. Because the filter protected the photo diode array from reflected laser light, withdrawal of the filter was impossible.

Tissue Ablation

Removal of the irradiated vascular tissue was achieved with the same precision as described previously by others\textsuperscript{11,14,15} and by us.\textsuperscript{16,17} Gross examination demonstrated no evidence of charring in any laser irradiated segment. Tissue ablation was limited to the target of the laser beam and light microscopy showed minimal laser-induced injury to the "nontarget" tissue. This minimal injury appeared as a borderline layer of slight discoloration. The cut edges of laser ablations were sharply demarcated, and the laser crater was delineated by a smooth surface (Figure 7). Energy fluence of 5 J/cm\textsuperscript{2}/pulse maintained a level of high-ablation quality similar to that described for lower energy fluence.\textsuperscript{11,16} Morphometric measurements of laser lesions revealed an ablation rate of 29.4 ± 5.2 μm/pulse for normal arterial components and noncalcified atheromas and 8.9 ± 2.3 μm/pulse for severely calcified lesions.

Discussion

The results of this study indicate that simultaneous identification and ablation of human vascular tissue by excimer laser energy is feasible in vitro. The principle of substance analysis by means of fluorescence spectroscopy is applied today in physics and biochemistry. It has been reported recently that identification of different tissue structures by means of laser-excited fluorescence spectroscopy is feasible with excimer (308 nm), nitrogen, argon, and the helium-cadmium lasers.\textsuperscript{20} Deckelbaum et al\textsuperscript{21} and Sartori et al\textsuperscript{22} have demonstrated discrimination of atherosclerotic from normal aortic and coronary artery segments by intima surface fluorescence analysis using low-power, pulsed nitrogen and argon lasers. The present study evaluates simultaneous ablation and detection of different human arterial wall layers with normal and pathological structure in real-time mode for the first time.

Previous in vitro studies have demonstrated that laser irradiation is able to remove severe arterial obstructions and occlusions with subsequently improved luminal patency.\textsuperscript{23–26} However, a high incidence of arterial perforation has been associated with laser angioplasty techniques in in vivo studies.\textsuperscript{4–9} Isner et al\textsuperscript{27} have shown that branching...
points, hard plaques, and eccentric stenoses predispose to laser-induced arterial perforation in the coronary circulation. This complication is generally accredited to the lack of adequate laser beam control and guidance. If improperly guided, the beam will penetrate and perforate the artery because the laser cannot distinguish between the tissue layers that should or should not be removed. In small and delicate vessels, additional mechanical injury from the fiber tip will cause susceptibility to wall damage and even perforation.

Several control and guidance techniques have therefore been proposed and developed to minimize the risk of perforation. They include modification of fiber tips, \textsuperscript{7,28} specialized guiding catheters and wires, \textsuperscript{7} application of laser energy during pullback, \textsuperscript{6} angioscopy, \textsuperscript{4} and high-frequency two-dimensional echocardiography. \textsuperscript{27} These techniques rely primarily upon the coaxial adjustment of the laser beam to the irradiated arterial segment or upon the imaging of the fiber position. In addition, some investigators have tried to obtain selective plaque ablation by sensitizing atherosclerotic lesions with tetracyclines, thus resulting in enhanced laser energy absorption for certain wavelengths. \textsuperscript{29}

The principle of real-time, pulse-by-pulse tissue fluorescence identification with particular regard to atherosclerotic pathology is of potential use for laser beam control. Tissue aimed at by the laser beam may be classified before the next laser pulse is delivered. Fluorescence spectra obtained thereby provide information whether the tissue layer in the target is an atherosclerotic or a normal arterial component. This spectral “fingerprint” may be used to develop a computer algorithm for feedback control that decides whether or not to ablate. Consequently, tissue ablation may be limited to atherosclerotic wall layers only, thereby minimizing the risk of perforation.

Differences in spectral behavior between tissue with and without calcium deposits are quite obvious in our studies. The strong-line emission of calcified layers throughout the visible wavelengths displays characteristic features of an atomar-line spectrum. Arterial samples that do not contain a prominent inorganic constituent, such as calcium, are characterized by broad-continuum spectra, which are indicative of complex organic constituents. All normal arterial tissue layers and plaque tissue without calcium revealed this type of spectral morphology. It is characterized by broad-based peak emission maxima with low intensity, and it contrasts quite clearly with the high-intensity, narrow-based, multipeakline spectrum of calcium. Tissue differentiation in the group of broad-continuum spectra is provided by different quantitative dimensions of fluorescence emission at wavelengths of 370 and 460 nm. The constitution of typical “complex” plaques, with fibrous components and extracellular lipids, which are not found in the normal arterial wall is considered responsible for the “plaque spectrum” of noncalcified lesions. Atherosclerotic lesions lack layers of well-

\begin{table}[h]
\centering
\caption{Media Type Fluorescence Spectra Preceding Perforation in Atherosclerotic Artery Segments}
\begin{tabular}{|c|c|}
\hline
Perforation (n) & Spectra suggestive of medial layers (n) \\
\hline
1 & 10 \\
2 & 30 \\
3 & 28 \\
4 & 26 \\
5 & 12 \\
6 & 29 \\
7 & 25 \\
8 & 11 \\
9 & 12 \\
10 & 12 \\
11 & 13 \\
12 & 25 \\
\hline
\end{tabular}
\end{table}
differentiated smooth muscle cells, and this may contribute to the approximately one-half reduction in intensity at 460 nm. Increased sensitivity of tissue differentiation may be achieved by means of enhanced spectral resolution, time-gating, and an alternative activating wavelength (308 nm).

As discussed by Deckelbaum et al., the choice of activating laser wavelength influences the fluorescence range and intensity ratio of peak emission. Their studies and studies by Sartori et al. and Kitrell et al. have investigated laser-induced arterial fluorescence with a different experimental setup were able to distinguish between normal arterial and atherosclerotic plaques. The fluorescence range and spectral morphology observed were different from our observations.

The question of whether the fluorescence emitted is generated by the molecules during degradation, by the degradation products themselves (plasma emission), or by the underlying intact molecules was not investigated in this study. The broad-continuum emission of fluorescence spectra may suggest that it is emitted from excimer laser-excited, but nondegraded, molecules in the borderline layer of the ablation. Further studies with activating energy fluence well below the ablation threshold will provide more accurate information about this particular question.

A main goal of this investigation was to determine whether wall perforation of atherosclerotic, arterial segments irradiated and penetrated by perpendicular beams could be prevented by correct and well-timed detection of fluorescence. Human advanced atherosclerosis is associated with extensive loss of medial smooth muscle cells. Atherosclerotic tissue layers are surrounded by the thinned media and the naturally small adventitia. Therefore, it is the thinned media layer that must be detected by the fluorescence analysis if wall perforation is to be prevented. All experiments leading ultimately to vessel perforation exhibited at least 10 subsequent spectra of “normal” pattern corresponding histologically to a thinned media. Provided that there is feedback control, this seems to be an appropriate safety layer for preventing perforation in a diseased peripheral circulation. However, in the coronary circulation, a “limited margin for error” between the target of vaporization and the underlying arterial wall may be present.

Fluorescence spectra in this study were obtained from irradiation strictly perpendicular to circumferential wall layers. As a result of circular tissue layer arrangement and perpendicular ablation, only one distinct layer was the target of ablation, and spectral analysis provided information about this particular layer. Therefore, these spectra are considered to be “pure” spectra. If the direction of irradiation is other than perpendicular, the cutting profile may cross different wall layers simultaneously, and “mixed” spectra can be expected, yielding information about two or even three different tissue compartments.

Further investigations will be necessary to evaluate mixed fluorescence spectra obtained from two or more tissue layers simultaneously.

Results of this investigation were obtained in a gaseous (air) interface medium. In an in vivo application of laser-excited fluorescence spectroscopy, a liquid interface medium of blood or saline solution would be likely. Problems may arise from difficult transmission of ultraviolet laser pulses through liquids. The use of optical fibers would, however, allow direct contact ablation, thereby minimizing the energy loss from transmission through interface media. Interaction with blood will play an important role, and hemoglobin emission is expected, which may change the characteristic spectral feature of tissue fluorescence. Although this aspect needs further investigation, the problem should be easily overcome by eliminating the hemoglobin fluorescence as a background signal.

In conclusion, irradiation-induced ablation by 248-nm excimer laser causes tissue fluorescence emission in human arteries with and without atherosclerotic injury. Real-time spectral analysis of this emission provides the option of characterizing tissue in the target of the laser beam, pulse by pulse, because it has the capacity to assess tissue composition from fluorescence spectra. This spectral behavior serves as an “optical fingerprint” of tissue that has been ablated by the fluorescence-exiting laser pulse. According to these findings, simultaneous ablation and identification of human arterial wall layers is feasible, thereby limiting laser ablation to diseased arterial layers, sparing healthy structures, and avoiding perforations. However, caution is required in extrapolating results obtained under strictly standardized laboratory conditions to the clinical setting.

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References


**Key Words** · laser angioplasty · fluorescence spectroscopy · arterial perforation
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