エキシマレーザーの人工血管に対する効果：人工血管閉塞に対するレーザー治療の基礎的研究

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Effects of Excimer Laser on Vascular Prosthetic Materials: A Preliminary Study for the Laser Treatment of Prosthetic Graft Failure

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要 旨
目的: 臨床使用上の安全性に対する基礎的検討として, in-vitro でエキシマレーザーの人工血管及び縫合糸に対する効果を検討した。
方法: XeCl エキシマレーザー, 波長 308 nm, パルス幅 130 nsec をコア径 600 µm のシリカファイバーを用いて照射した。繰り返し周波数は 5 Hz で, 臨床に使用されるフルーエンス (35 と 50 mJ/mm2) を選択した。Expanded polytetrafluoroethylene (PTFE) と Dacron の人工血管, 及びボリプロビレンと PTFE 糸に in vitro でレーザー照射し, その耐性を観察した。これらの人工血管を犬の動脈に移植し, 18 週後に摘出した。内膜肥厚部, 器質化血栓, 及び対照として犬の大腿動脈を用いた。標本の厚さを, それを貫通するのに有したパルス数で除したものをアブレーションレートとして, エキシマレーザーに対する標本の耐性を定量化した。縫合糸の耐性評価には, それを破綻させるのに要するパルス数を測定した。
結果: アブレーションレート (μm/pulse, mean±SD) は 35 mJ/mm2 で: 大腿動脈 11.7±2.00, 器質化血栓 10.5±3.50, 内膜肥厚部 4.02±1.02†, 摘出 PTFE グラフト 4.44±0.01†, 摘出 woven Dacron 4.15±0.24†, 摘出 knitted Dacron 3.00±0.42†, 50 mJ/mm2 では: 大腿動脈 47.0±7.29, 器質化血栓 26.7±5.00†, 内膜肥厚部 11.0±3.00†, 摘出 PTFE グラフト 0.94±0.03†, 摘出 woven Dacron 13.2±0.70†, 摘出 knitted Dacron 8.33±1.00† (大腿動脈と比べ p<0.01)。両方のフルーエンスともボリプロビレン糸は 40 パルス以内で切断されるが PTFE 糸は 2000 パルスでも切断されなかった。
結論: エキシマレーザーは PTFE グラフト閉塞に使用するには, 人工血管には重大なダメージは生じないこと, しかしボリプロビレン糸はレーザーに対し極めて脆弱であることが示唆された。

キーワード: エキシマレーザー血管形成術, 人工血管, 血管縫合糸
Results: The ablation rates (μm/pulse, mean±SD) were as follows at 35 mJ/mm²: femoral artery 11.7±2.00, organized thrombus μperforate the sample was defined as the ablation rate to quantify the resistance of the graft samples to excimer laser energy. The ratio of sample thickness divided by the number of pulses required to perforate the sample was defined as the ablation rate to quantitate the resistance of the graft samples to excimer laser energy. The number of laser pulses required for suture disruption was counted to evaluate the durability of suture materials.

Purpose: We studied the in-vitro effects of the excimer laser on vascular prostheses and sutures as a preliminary determination of safety prior to clinical use.

Methods: XeCl excimer laser energy, wavelength 308 nm with 130 nsec pulse width, was delivered via a 600 μm core silica fiber. A repetition rate of 5 Hz and clinically relevant fluences (35 and 50 mJ/mm²) were selected. Expanded polytetrafluoroethylene (PTFE) and Dacron grafts, and polypropylene and PTFE sutures were exposed to laser radiation in vitro to observe their resistance. Both grafts were interposed in canine arteries, and explanted after 18 weeks. Sections of intimal hyperplasia, organized thrombus, and control canine femoral arteries were studied. The ratio of sample thickness divided by the number of pulses required to perforate the sample was defined as the ablation rate to quantitate the resistance of the graft samples to excimer laser energy. The number of laser pulses required for suture disruption was counted to evaluate the durability of suture materials.

Results: The ablation rates (μm/pulse, mean±SD) were as follows at 35 mJ/mm²: femoral artery 11.7±2.00, organized thrombus 10.5±3.50, hyperplastic intima 4.02±1.02‡, explanted PTFE graft 0.44±0.01†, explanted woven Dacron 4.15±0.24‡, explanted knitted Dacron 3.00±0.42‡. At 50 mJ/mm²: femoral artery 47.0±7.29, organized thrombus 26.7±5.00†, hyperplastic intima 11.0±3.00‡, explanted PTFE graft 0.9±0.03†, explanted woven Dacron 13.2±0.70‡, explanted knitted Dacron 8.33±1.00†. (‡smaller than femoral artery [p<0.01].) Fourteen pulses were enough to cut the polypropylene suture at both energy densities, whereas even 2000 pulses did not break the PTFE sutures.

Conclusions: These results indicate that the excimer laser, if used in treatment of PTFE graft failure, will not significantly damage the prosthesis; however, polypropylene suture is extremely vulnerable.

Key words: excimer laser angioplasty, vascular prosthesis, vascular suture

1. INTRODUCTION

Patients with severe manifestations of lower extremity arterial occlusive disease often require peripheral bypass surgery for limb salvage and preservation of function. Synthetic conduits, expanded polytetrafluoroethylene (PTFE) and polyethylene terephthalates (Dacron), are routinely used for bypass surgery above the knee, however, infrainguinal graft failure occurs in 40% to 60% at 5 years1-3. Although undergoing revision procedures (thrombolysis, thrombectomy, balloon angioplasty, or insertion of a new bypass graft), the overall prognosis for limb salvage in patients with failed infrainguinal bypass graft is poor4.

Laser angioplasty, undergoing clinical trial for peripheral and coronary artery recanalization, may also have potential for the treatment of prosthetic graft failure. No laser irradiation technique, however, has achieved precision in controlling direction and depth of injury. Directing the laser over a guide wire is still the standard approach for laser angioplasty. The effect of thermal lasers on prosthetic grafts or sutures can be predicted based on the melting point of the material, however, there is no such data for the ultra violet excimer laser which ablates tissue by a photochemical reaction.

The purpose of this study was to evaluate the effects of excimer laser on vascular prostheses and sutures in order to determine the safety of excimer laser application. The resistance of commonly used prosthetic grafts (PTFE and Dacron) and sutures (polypropylene and PTFE) to damage by laser radiation was investigated. The resistance of canine femoral arteries, organized thrombus and hyperplastic intima was determined for comparison.

2. MATERIALS AND METHODS

2.1 Samples

PTFE (Impra, Inc., Tempe, Ariz.), Cooly Veri-Soft woven Dacron (Meadow Medicals, Inc., Oakland, N.J.), and Microvel Double Velour knitted Dacron (Meadow Medicals, Inc., Oakland, N.J.) were selected for study. Twenty segments each of PTFE, woven and knitted Dacron grafts, and the same number and type of the samples obtained from canines after 18 weeks of implantation were used. Four occluded PTFE grafts were harvested from the carotid or femoral arteries of four mongrel dogs. The explanted PTFE graft samples, the organized thrombi and the segments of intimal hyperplasia were obtained from those occluded PTFE grafts. We performed patch-plasty of the femoral arteries of four mongrel dogs using woven and knitted Dacron and harvested the prosthetic materials after 18 weeks. The anesthesia and the management of the experimental animals were as reported previously5. The animal care complied with the "Principles of Laboratory Animal Care" (formulated by the National Society for Medical Research) and the Guide for the Care and Use of Laboratory Animals (NIH Publication No.86-23, revised 1985).

The sutures studied were 20 samples each of polypropylene: Prolene (Ethicon, Inc., Somerville, N.J.) and PTFE: Gore-Tex (W.L. Gore & Associates, Flagstaff, Ariz.) sutures. We selected 6-0 Prolene and CV-8 Gore-Tex since their diameters were almost the same.

2.2 Treatment of samples before laser irradiation

All grafts and specimens (organized thrombus, sections of anastomotic intimal hyperplasia, and femoral arteries) were cut into square segments before laser irradiation. Twenty segments of each graft and explanted graft were prepared.
Twenty samples of femoral artery, 8 segments of organized thrombus and hyperplastic intima were obtained from the experimental animals. The thickness of the sample was measured with a micrometer (Outside micrometer No. 101-103, Mitutoyo Mfg. Co. Ltd., Tokyo, Japan) before irradiation. These samples were placed into a quartz grass container and submerged in saline solution.

2.3 Laser and delivery systems

Energy from a xenon chloride (XeCl) excimer laser (Advanced Interventional Systems, Inc., Irvine, Calif.) operating at 308 nm wavelength with 130 ns pulse width was delivered through a 600 µm core silica fiber. A repetition rate of 5 Hz frequency allowed us to count the number of pulses. With higher frequency (e.g. 20 Hz), it was impossible to count the exact number of pulses which were required to perforate the sample. Output energy densities of 35 and 50 mJ/mm² were selected because they are the most likely to be used clinically.

2.4 Methods of laser irradiation

The laser probe was applied perpendicularly against the luminal surface of the materials in saline solution (35 - 37°C) with a constant force of 4 gm/mm² (Fig.1). The samples were irradiated until perforation occurred, and the number of pulses required for graft perforation was recorded. We defined that the sample was perforated when a violet spot was seen clearly on the paper placed underneath the sample container. The ratio of the known sample thickness divided by the number of pulses required to perforate the sample was defined as the "ablation rate". A high ablation rate indicates a low tolerance of the substance being tested to laser irradiation.

Using the experimental apparatus shown in Fig.2, the laser was directed against sutures in a saline bath at 35 - 37°C. Non-contact irradiation from a distance of 1 mm was used to test the sutures. The number of pulses required for suture disruption up to 2000 was recorded.

2.5 Morphological analysis

Additional samples were irradiated for microscopic observations. Ten, 100, 300 and 1000 pulses were given to each sample with a frequency of 5 Hz and the energy density of 50 mJ/mm². Following irradiation, the non-implanted samples were mounted on studs and coated with gold palladium to be visualized with a Hitachi S405 A-2 scanning electron microscope. The explanted samples were fixed with 10% formalin, embedded in paraffin, cut through the damage crater parallel to the axis of the laser beam, stained with hematoxylin-eosin, and examined with an Olympus BX50 light microscope.

2.6 Statistical analysis

All values are expressed as mean±the standard deviation (SD). The significance of differences among the ablation rates were assessed by the Dunnett's test following one way analysis of variance. The significance of differences between the resistances of the sutures were assessed by the Student's t-test (unpaired). Differences were considered significant if the p value was less than 0.05.

3. RESULTS

3.1 Graft materials

The ablation rates (μm/pulse) at 35 and 50 mJ/mm² energy density for the three tissues, three grafts and three explanted grafts are shown in Table 1. All ablation rates at 50 millijoules were higher than at 35 millijoules as expected. In the plain non-implanted graft groups, PTFE showed remarkable tolerance to the excimer laser. Dacron grafts, especially knitted Dacron showed little resistance to laser irradiation. After implantation, the PTFE became weaker and the Dacron became stronger. All explanted graft materials were significantly stronger than the femoral artery at both energy densities. PTFE grafts became less resistant after implantation but were still 50 times more resistant than the femoral artery,
20 times more than the organized clot and 10 times more than the hyperplastic intima.

With an energy density of 50 mJ/mm² and a constant force of 4 gm/mm², only 10 pulses were required to perforate the Dacron graft (Fig.3,A). In contrast even 100 pulses made only a hollow on the PTFE graft (Fig.3,B). After irradiation the graft surfaces were generally smooth. A "string of beads" appearance could be seen on the tips of the fibers around the perforation of the Dacron graft probably representing thermal damage.

Tissue incorporation made the Dacron graft stronger but 100 pulses were enough to perforate (Fig.4,A). The PTFE became less resistant after implantation but 300 pulses made only a concave on the graft (Fig.4,B). Carbonization, a characteristic appearance of thermal laser irradiation, was not observed in any specimen in this study.

Table 1 Ablation rates* for graft materials, femoral artery, thrombus and hyperplastic intima (mean±SD).

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Energy Density (mJ/mm²)</th>
<th>35</th>
<th>50</th>
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<tbody>
<tr>
<td>Femoral artery (n=20)</td>
<td>11.7±2.00</td>
<td>47.0±7.29</td>
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<tr>
<td>Organized thrombus (n=8)</td>
<td>10.5±3.50</td>
<td>26.7±5.00†</td>
<td></td>
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<tr>
<td>Hyperplastic intima (n=8)</td>
<td>4.02±1.02†</td>
<td>11.0±3.00†</td>
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<table>
<thead>
<tr>
<th>Grafts</th>
<th>Energy Density (mJ/mm²)</th>
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<th>50</th>
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<tr>
<td>PTFE (n=20)</td>
<td>0.04±0.01†</td>
<td>0.06±0.01†</td>
<td></td>
</tr>
<tr>
<td>Woven Dacron (n=20)</td>
<td>13.7±1.91**</td>
<td>19.7±3.30†</td>
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</tr>
<tr>
<td>Knitted Dacron (n=20)</td>
<td>43.9±5.45**</td>
<td>56.0±5.05**</td>
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</table>

<table>
<thead>
<tr>
<th>Explanted grafts</th>
<th>Energy Density (mJ/mm²)</th>
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<tr>
<td>PTFE (n=20)</td>
<td>0.44±0.01†</td>
<td>0.94±0.03†</td>
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<tr>
<td>Woven Dacron (n=20)</td>
<td>4.15±0.24†</td>
<td>13.2±0.70†</td>
<td></td>
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<tr>
<td>Knitted Dacron (n=20)</td>
<td>3.00±0.42†</td>
<td>8.33±1.00†</td>
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</table>

* The ratio of the known sample thickness divided by the number of pulses required to perforate the sample. A low ablation rate indicates high resistance of the material tested to laser energy.
** Larger/smaller than femoral artery (p=0.01, Student's t test).

Fig.3 Scanning electron micrographs of the plain grafts after laser irradiation (original magnification x 70). A, Dacron graft after receiving 10 pulses of excimer laser (output energy density: 50 mJ/mm², applied stress: 4 gm/mm²). B, PTFE graft after receiving 1000 pulses of excimer laser (output energy density: 50 mJ/mm², applied stress: 4 gm/mm²).

Fig.4 The light micrographs of the cross sections of the explanted grafts after laser irradiation (hematoxylin-eosin stain; original magnification x 40). A, Dacron graft after receiving 100 pulses of excimer laser (output energy density: 50 mJ/mm², applied stress: 4 gm/mm²). B, PTFE graft after receiving 300 pulses of excimer laser (output energy density: 50 mJ/mm², applied stress: 4 gm/mm²).

Table 2 Pulses required for suture disruption* (mean±SD)

<table>
<thead>
<tr>
<th>Suture</th>
<th>Energy Density (mJ/mm²)</th>
<th>35</th>
<th>50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polypropylene (n=20) (Prolene 6-0) [diameter 0.10±0.002mm]</td>
<td>12.6±1.34</td>
<td>10.8±0.84</td>
<td></td>
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<tr>
<td>PTFE (n=20) (Gore-Tex CV-8) [diameter 0.098±0.005mm]</td>
<td>&gt;2000**</td>
<td>&gt;2000**</td>
<td></td>
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</table>

* 5 gm tension, fiber distance from sample surface: 1.0 mm.
*** p<0.01 versus polypropylene (Student’s t test).
Diameter of the sutures were measured by the outside micrometer.

Fig.5 The scanning electron micrographs of the plain sutures after laser irradiation (original magnification x 40). A, Polypropylene suture broke with 10 pulses of excimer laser (output energy density: 50 mJ/mm², fiber distance from sample surface: 1.0 mm). B, PTFE suture after receiving 1000 pulses of excimer laser (output energy density: 50 mJ/mm², fiber distance from sample surface: 1.0 mm).
3.2 Suture materials

Table 2 shows the number of pulses required for suture disruption. PTFE suture was very resistant compared to the easy destroyed polypropylene suture. Fourteen pulses were enough to cut the polypropylene suture at both energy densities, whereas even 2000 pulses did not break the PTFE suture.

The polypropylene suture, broken with 10 pulses at 50 mJ/mm², showed a sharp cutting surface (Fig.5,A) which is not seen with thermal laser irradiation. One thousand pulses at the same energy density only depressed the PTFE suture without cutting (Fig.5,B).

4. DISCUSSION

Laser angioplasty offers theoretical advantages over percutaneous transluminal balloon angioplasty (PTBA)\(^6,7\). Laser vaporization of atherosclerotic tissue leaves a smoother, less thrombogenic surface than that seen with PTBA\(^6,8,9\).

Ablation of old organized thrombi may be useful for the adjunctive treatment of anastomotic and distal occlusive disease. Laser thermal probe recanalization for graft failure has already performed experimentally\(^10\) and clinically\(^11,12\), and although the early results were somewhat encouraging, patency rates at one year were disappointing\(^13,14\).

Clinical trials of excimer laser have shown feasibility in treatment of both peripheral and coronary artery disease\(^15-18\). The different mechanism of action of the excimer laser\(^15,19,20\) as opposed to conventional thermal lasers\(^8,25\) also positions this devise as a potentially useful tool in salvage of the failing graft. The technique of laser ablation, however, even with the excimer laser is not precise enough at the present time to accurately control direction and depth of injury. Therefore the effect of the excimer laser on vascular prosthetic materials must be known before clinical application of excimer laser angioplasty for graft failure to eliminate the risk of perforation.

The ablation mechanism of the ultraviolet excimer laser is fundamentally different from conventional thermal lasers\(^21,22\). Absorbed ultraviolet photons disrupt molecular bonds, removing tissue by photochemical rather than thermal mechanisms. Thus, the effects of the excimer laser on materials cannot be estimated from their melting points.

PTFE grafts and PTFE sutures showed excellent resistance both before and after implantation. Plain Dacron grafts were weaker than PTFE to the laser irradiation. Although the melting point of PTFE (327°C) is higher than that of Dacron (260°C), we think the main reason of the difference in excimer laser durability is not heat-resisting property, judging from the appearances of the samples after irradiation as mentioned later. The strong chemical bonds characteristic of PTFE may be the reason for this remarkable durability, or the high reflectivity of PTFE may protect graft material against high density photons of the excimer laser.

Tissue incorporation lessened the resistance of PTFE somewhat, but paradoxically Dacron became stronger. Tissue incorporation may reduce the reflectivity of PTFE and increase the ablation rate. On the other hand, as Dacron has a higher porosity at implantation and allows greater tissue incorporation, this rich tissue ingrowth may protect graft material from laser photons. Although explanted Dacron was stronger than femoral artery, excimer laser irradiation may cause fiber disruption of Dacron grafts and possibly lead to late aneurysm formation.

The morphologic appearances of the samples after exposure to the excimer laser were different than that seen with conventional lasers\(^8,25\). Thermal effect was observed in only the tips of Dacron fibers around the site of perforation. From this in vitro observation, we suspect that the endoluminal surface after excimer laser ablation may be smoother and less thrombogenic than the charred surface remaining after use of thermal lasers.

The results of the excimer laser on sutures indicate that laser ablation for anastomotic lesions in which polypropylene sutures have been used may be contraindicated. The integrity of an anastomosis depends on the graft material and the suture. The fracture of the suture does not always cause acute disruption of the anastomosis, but it might lead to late pseudoaneurysm formation. Our results also suggest that the anastomotic intimal hyperplasia associated with PTFE grafts in which the PTFE sutures have been used may be a possible application for the excimer laser.

We could not use explanted sutures because of the difficulty in harvesting a sufficiently long segment of suture. Difference in the effects of the excimer laser on plain and implanted sutures should not be great since these monofilament sutures are high density and do not allow tissue ingrowth into the suture materials\(^20\).

We conclude that excimer laser irradiation is safe for use in prosthetic graft failure (especially PTFE grafts), however, the presence of polypropylene suture should be regarded as a distinct hazard.

**REFERENCES**


