Spatially selective photocoagulation
of biological tissues: feasibility
study utilizing cryogen spray cooling

Bahman Anvari, B. Samuel Tanenbaum, Thomas E. Milner, Kimberly Tang, Lih-Huai Liaw,
Ken Kalafus, Sol Kimel, and J. Stuart Nelson

Successful laser treatment of selected dermatoses such as hemangiomas requires thermally induced
damage to blood vessels while protecting the epidermis. We present and test a procedure in a rabbit
liver tissue model that utilizes cryogen spray cooling during continuous Nd:YAG laser irradiation to
induce deep photocoagulation necrosis while protecting superficial tissues from thermal injury. Gross
and histologic observations are consistent with calculated thicknesses of protected and photocoagulated
tissues and demonstrate the feasibility of inducing spatially selective photocoagulation when cryogen
spray cooling is used in conjunction with laser irradiation. This procedure may be useful in the
thermal treatment of some pathological conditions for which it is desired that deep photocoagulation be
induced while protecting superficial tissues.

Key words: Chlorodifluoromethane, hemangioma, infrared radiometry, laser, microwave, thermal-
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1. Introduction

Various thermally mediated therapeutic procedures
utilizing microwave, infrared, or visible electromagnetic
radiation have become of interest to many
investigators in recent years.1–3 In many of these
therapeutic procedures the objective is to induce
coagulation necrosis of certain tissue components
while protecting superficial tissues from thermal
injury. For example, successful laser treatment of
dermatoses such as port-wine stain lesions, heman-
ghiomas, and telangiectasias is based on the photoco-
agulation of blood vessels without inducing thermal
injury to the overlaying epidermis and papillary
dermis, which could result in skin-surface textural
changes or scarring.4–8

Cryogen spray cooling is a potentially effective
method for protecting superficial tissues from ther-
mal injury.9 By the application of short-duration
spurt of a cryogen (of the order of milliseconds), rapid
and selective cooling of tissue is possible: superfi-
cial tissues are cooled while the temperature of
deeper tissues remain unchanged.9 Evaporation of
the cryogen on the surface provides the mechanism
for rapid removal of heat from the tissue. For
example, when tetrafluoroethane (boiling point
≈ −26 °C) is used as a cryogen, surface-temperature
drops of the order of 30–40 °C have been obtained
within 5–100 ms.9–11 Theoretical results indicate
that spray cooling the skin with tetrafluoro-
ethane just prior to pulsed-laser irradiation can
selectively cool the epidermis and yet permit photo-
coagulation of diluted port-wine stain blood ves-
sels.12,13 Successful blanching of port-wine stain
lesions without either epidermal thermal injury or
skin-surface textural changes has been reported
when the skin is precooled with tetrafluoroethane
immediately prior to flash-lamp-pumped pulsed-dye-
laser irradiation with a relatively high light dosage
(e.g., 10 J/cm²).10,11,13
In this paper we present the results of an experiment using continuous Nd:YAG laser irradiation and cryogen spray cooling. The Nd:YAG laser (λ = 1064 nm) light is poorly absorbed by biological tissues and penetrates several millimeters into tissues to achieve deep photocogulation, while the cryogen spray cooling protects against thermal damage to superficial tissues. A theoretical analysis of the tissue thermal response to continuous laser irradiation in the presence of cryogen spray cooling is presented, and clinical implications of this procedure for the treatment of hemangiomas are discussed.

2. Materials and Methods

Light emitted from a Nd:YAG laser (LaserSonic Inc., Santa Clara, Calif.) was used as a heat source to induce deep tissue photocogulation in freshly excised rabbit liver. Although liver is a relatively homogeneous medium (unlike skin), it nevertheless serves as a convenient tissue phantom in which photocogulation necrosis can be clearly observed.

The experimental apparatus for laser irradiation in conjunction with cryogen spray cooling is shown in Fig. 1. Laser light delivered through a 600-µm fiber directly incident onto the liver sample surface. Various irradiation parameter sets (Table 1) were selected to cover a relatively wide range. Irradiation times of the order of seconds were used to achieve a large volume of photocoagulated tissue. Each parameter set was repeated at least three times with little variation in results.

Chlorodifluoromethane (boiling point of ≈ −40 °C) (Aldrich Chemical Company, Milwaukee, Wisc.), a nonflammable refrigerant, was sprayed onto the liver through an electronically controlled standard automobile fuel-injection solenoid valve positioned 4 cm from the sample surface at a 30° angle from the tissue normal. The time duration of the cryogen spurt was fixed at 50 ms by a programmable digital delay generator (Model DG535, Stanford Research Systems, Sunnyvale, Calif.). The cooled site on the tissue surface was concentric with the laser-irradiated site and ~10 mm in diameter.

Radiometric measurement of the surface temperature at the center of the laser-irradiated site was used to trigger the delivery of cryogen spurts. When the radiometric surface temperature reached a prespecified threshold value (30 °C), a cryogen spurt of 50-ms duration was delivered onto the liver surface. In this way, repetitive, pulsed cryogen spray cooling during continuous laser irradiation was accomplished.

Infrared emission from the liver surface was detected by the use of a 1-mm² liquid-N₂-cooled HgCdTe detector (Model MDD-10E0-S1, Cincinnati Electronics, Mason, Oh.), optically filtered at the cold stop by a 10.6–14-µm bandpass filter. Because the infrared absorption coefficient of water in this range is approximately 60 mm⁻¹ (Ref. 14), we expect that contributions to the infrared signal originate predominantly from superficial tissues (approximately the uppermost 15 µm).

The detector was placed at the focal plane of a 25-mm-diameter 1/1 Ge lens configured for unit magnification. For an improved signal-to-noise ratio, the pupil was stopped to a 5-mm diameter and the infrared radiation was amplitude modulated (3.5 kHz) by the use of a chopper (Model SR540, Stanford Research Systems) and synchronously detected by a lock-in amplifier (Model SR550, Stanford Research Systems). The output signal of the lock-in amplifier was used as the threshold trigger for the digital delay generator.

The infrared detection system was calibrated for temperature changes above and below the ambient value (23 °C). The lock-in amplifier output voltage was measured as a function of the surface temperature of (1) an aluminum block coated with highly emissive (ε = 0.97) black paint (TC-303 black, GIE Corporation, Provo, Ut.) and heated by a resistive element from 23 °C to 75 °C, and (2) a thermoelectric cooler (ITI FerroTech, Chelmsford, Mass.) that was coated with the same black paint and cooled from 23 °C to −20 °C. The surface temperature of the aluminum block and the thermoelectric cooler was

Table 1. Laser-Irradiation Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Laser Power</th>
<th>Irradiation Time</th>
<th>Spot Diameter</th>
<th>Irradiance</th>
</tr>
</thead>
<tbody>
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<td>W</td>
<td>s</td>
<td>mm</td>
<td>kW/m²</td>
</tr>
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<td>5, 10</td>
<td>120, 90</td>
<td>7</td>
<td>130, 260</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>10, 15, 20</td>
<td>7</td>
<td>520</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>10</td>
<td>3</td>
<td>2,830</td>
</tr>
<tr>
<td>4</td>
<td>40, 50</td>
<td>30, 15</td>
<td>9</td>
<td>630, 790</td>
</tr>
</tbody>
</table>

Fig. 1. Schematic diagram of the experimental apparatus for cryogen spray cooling and Nd:YAG laser irradiation measurement of surface temperature during the experiment is made by IR radiometry.
measured by the use of a precision thermistor (Model 8681, Keithley Instruments, Cleveland, Ohio). The radiometric temperature was computed from calibration data by use of a polynomial fit.

3. Results and Discussion

In Fig. 2 we present a cross section of rabbit liver tissue dissected immediately after the procedure along the central axis of laser irradiation (parameter set 2, \( t_{\text{irrad}} = 15 \) s). Photocoagulated tissue (\( \approx 4 \) mm thick, 5-mm maximum width) below a protected layer (\( \approx 400 \) µm thick), between the arrows, is observed. Photocoagulation was accompanied by an observable loss of the natural red color and whitening of the tissue, indicative of optical-property changes, in part caused by thermal denaturation of hemoglobin molecules. In the absence of cryogen spray cooling there was no protected layer, and the region of photocoagulation extended from the surface deep into tissue.

Histologic sections of the nonirradiated and irradiated (parameter set 2, \( t_{\text{irrad}} = 10 \) s)-cooled rabbit liver tissue are shown in Figs. 3. The nonirradiated section [Fig. 3(a)] shows the red blood cells appearing as small dark regions within blood vessels and capillaries, which appear as white. A confined zone of photocoagulation is observed in the irradiated and cryogen-spray-cooled section [Fig. 3(b)]. Red blood cells are still present in superficial tissue (indicated by the arrow), but they are completely absent in the deeper regions because of photocoagulation of the blood vessels and capillaries. The photocoagulated tissue is more compact when compared with the protected superficial and the nonirradiated tissues, an indication that thermal destruction of blood vessels and capillaries has taken place.

Photocoagulation necrosis without damage to the superficial tissues was also achieved with parameter sets 1 and 4. Irradiation times in parameter set 1 (90 and 120 s), however, may be considered too long for clinical use. Protection of the superficial tissues was not achieved with parameter set 3. When the laser beam was focused to a small spot (3-mm diameter), the resulting irradiance was so large (2830 kW/m²) that the amount of heat generated was greater than that which could be removed by cooling.

An example of the recorded radiometric surface temperature in response to laser irradiation (parameter set 2, \( t_{\text{irrad}} = 15 \) s) and repetitive cryogen spray cooling is shown in Fig. 4. The radiometric surface temperature shows cycles of laser-induced temperature increases to 30 °C, the prespecified threshold temperature for the application of cooling, followed by rapid temperature reductions to approximately −5 °C after cryogen is sprayed onto the tissue surface. The frequency of cryogen spurts increases toward the end of the irradiation time, indicating that more heat is diffusing toward the surface as the internal temperature of the liver sample increases.

Temperature distributions within the tissue during continuous laser irradiation and repetitive pulsed cooling can be calculated by the solution of the

![Fig. 2. Gross cross section of rabbit liver tissue irradiated with Nd:YAG laser (parameter set 2, \( t_{\text{irrad}} = 15 \) s) repetitively cooled with 50-ms chlorodifluoromethane spurts. The protected region is indicated by the region between the arrows. The surface of the tissue is not visible in the figure.](image1)

![Fig. 3. Histologic sections of (a) nonirradiated, and (b) irradiated tissue (parameters set 2, \( t_{\text{irrad}} = 10 \) s) while repetitively cooled with 50-ms chlorodifluoromethane spurts.](image2)
heat-conduction equation. In one dimension,

$$\frac{\partial^2 T(z, t)}{\partial z^2} + \frac{Q_L(z)}{k} = \frac{1}{\alpha} \frac{\partial T(z, t)}{\partial t},$$

(1)

where $T$ is the temperature, $z$ is the distance into the tissue with the origin at the tissue surface, $t$ is time, $\alpha$ is the thermal diffusivity ($2 \times 10^{-7}$ m$^2$/s for liver tissue), $k$ is the tissue thermal conductivity (0.59 W/mK), and $Q_L$ is the volumetric heat generation caused by laser irradiation. We assume that $Q_L$ is distributed as

$$Q_L(z) = \mu_a \phi_0 \exp(-\mu_a z),$$

(2)

where $\mu_a$ is the absorption coefficient of liver at 1064 nm (0.88 mm$^{-1}$) and $\phi_0$ in watts per meter squared, is the irradiance. Our simple assumption of the exponential decay of $Q_L$ with depth does not take into account the scattering of laser light within tissue. Backscattering of laser light is expected to cause the maximum value of $Q_L$ to occur just below the surface of the sample. Deep in the tissue, $Q_L$ is overestimated by the neglect of scattering. However, as a first approximation, the exponential model provides insight into the thermal response of tissue during irradiation in conjunction with cryogen spray cooling. The effect of blood perfusion on the resulting temperature distributions is not taken into account because this is an in vitro study. However, for in vivo studies and in highly vascularized tissues, contributions of blood perfusion to heat transfer can be significant for heating times, of the order of minutes.

We assume a periodic temperature variation (Fig. 4) and write a thermal boundary condition of the form

$$T(z = 0, t) = T_{\text{thresh}} - \frac{T_{\text{thresh}} - T_{\text{min}}}{2} \left[ 1 - \cos 2\pi ft \right].$$

(3)

Here, $T_{\text{thresh}}$ is the prespecified threshold temperature (30°C), $T_{\text{min}}$ is the minimum surface temperature achieved as a result of cooling (−5°C), and $f$ (Hz) is the spurt repetition rate.

The solution of Eq. (1) with boundary-condition equation (3) can be expressed as a superposition of the thermal responses caused by laser-induced heating and cryogen spray cooling:

$$T(z, t) = \Delta T_L(z, t) + \Delta T_C(z, t) + T_{\text{thresh}},$$

(4)

where

$$\Delta T_L = \frac{\phi_0}{\mu_a k} \left[ \text{erfc}(\tilde{z}) - \exp(-\mu_a \tilde{z}) \right] + \frac{\exp(-\tilde{z}^2)}{2} \left[ \text{erfc}(\mu_a \tilde{z}) - \text{erfc}(\mu_a + \tilde{z}) \right],$$

(5)

and

$$\Delta T_C = \frac{T_{\text{min}} - T_{\text{thresh}}}{2} \left[ \text{erfc}(\tilde{z}) - \exp(-\beta z \cos 2\pi ft - \beta z) \right],$$

(6)

where

$$\tilde{z} = z / 2 \sqrt{\alpha t}, \quad \beta = \sqrt{\pi f / \alpha},$$

(7)

and $\text{erfc}(x)$ defined as $\exp(x^2) \text{erfc}(x)$, where $\text{erfc}(x)$ is the complementary error function, $1 - \text{erf}(x)$.

The calculated temperature distributions in response to laser irradiation (5 W, 7-mm irradiated spot diameter) and cryogen spray cooling are shown in Fig. 5, where $f = 0.625$ Hz (from Fig. 4, it can be seen that there is a cryogen spurt released approximately every 1.6 s) and $\mu_a = 0.88$ mm$^{-1}$. From boundary-condition equation (3), it is seen that the surface temperature reaches a minimum when $t = n / f \pm 1 / 2f$ (with $n$ a positive integer) and a maximum when $t = n / f$. The solid and dashed curves shown in Fig. 5 correspond to the calculated temperature distributions when surface temperatures reach a minimum and a maximum, respectively. For example, when $n = 5$, the minimum and maximum surface temperatures occur at 7.2 and 8.0 s, respectively [Fig. 5a]. With the assumption that the threshold temperature for photocoagulation necrosis is approximately 55°C when this temperature is maintained for seconds, previous measurements of tissue temperature during continuous Nd:YAG laser irradiation have indicated that observable tissue whitening occurs at approximately 55°C (Ref. 26), superficial tissue (≈0.400 μm thick) remains below the threshold temperature required for photocoagulation (55°C), whereas deeper tissue is denatured. When $n = 10$, the minimum and maximum surface temperatures occur at 15.2 and 16.0 s, respectively [Fig. 5b]. The thickness of photocoagulated tissue
has increased, while the temperature of the superficial tissue still remains below 55°C.

The heat flux \( q \) [W/m²], removed at the surface is obtained by the superposition of fluxes arising from cooling and laser irradiation:

\[
q(t) = q_L(t) + q_C(t),
\]

where

\[
q_L(t) = k \frac{\partial T_L(z,t)}{\partial z} \bigg|_{z=0} = \phi_0 (1 - \text{erfc} \alpha),
\]

\[
q_C(t) = k \frac{\partial T_C(z,t)}{\partial z} \bigg|_{z=0} = k \left( \frac{T_{\text{min}} - T_{\text{thresh}}}{2} \right)
\]

\[
\times \left[ -\frac{1}{\sqrt{\pi \alpha t}} + \sqrt{2\beta} \cos \left( 2\pi ft + \frac{\pi}{4} \right) \right].
\]

The calculated values of the heat flux removed at the tissue surface in response to Nd:YAG laser irradiation (5 W, 7-mm irradiated spot diameter) and periodic cryogen-spray cooling at 0.625 Hz. The solid curves correspond to minimum surface temperatures at \( a \) \( t = 7.2 \) s, and \( b \) \( t = 15.2 \) s; the dashed curves correspond to maximum surface temperatures at \( a \) \( t = 8.0 \) s, and \( b \) \( t = 16.0 \) s.

Fig. 5. Calculated temperature distributions in response to Nd:YAG laser irradiation (5 W, 7-mm irradiated spot diameter) and periodic cryogen-spray cooling at 0.625 Hz.

Fig. 6. Calculated values of the heat flux removed at the tissue surface in response to Nd:YAG laser irradiation (5 W, 7-mm irradiated spot diameter) and periodic cryogen-spray cooling at 0.625 Hz.

The thickness of the protected superficial tissue decreases with the irradiation time [Fig. 7(b)]. With a laser power of 5 W delivered for 16 s, an approximately 300-µm thickness of tissue is protected, whereas an almost 4.2-mm thickness of the deeper tissue is photocoagulated. Although we have not accounted for laser-light scattering and changes in optical properties resulting from photocoagulation, the calculated thicknesses of the protected and photocoagulated tissues are consistent with the experimental results and indicate the feasibility of inducing deep photocoagulation while protecting the superficial tissue. Nevertheless, the thickness of the photocoagulated tissue is somewhat overestimated because we have neglected light scattering and the radial diffusion of heat, which would reduce the temperature of deeper tissues. Furthermore, this simple theoretical model cannot successfully be applied to experiments involving high values of \( \phi_0 \) because unrealistically high temperatures and much smaller protected layers than those found experimentally are predicted. A more realistic model should account for the utilization of laser energy in bringing about phase changes associated with thermal dena-
rable or greater temperature reductions. This advantage is evident in Fig. 4, from which it can be seen that the time-averaged surface-temperature reduction is \( \sim 16 \degree C \), which is probably somewhat larger than the surface-temperature drop when water or air cooling is used.\(^{19,32}\)

We are currently investigating the feasibility of inducing spatially selective coagulation using cryogen spray cooling in conjunction with laser irradiation for the treatment of hemangiomas and other selected dermatoses. The results of these studies will be reported in future papers.

4. Conclusions

We have experimentally and theoretically demonstrated the feasibility of inducing spatially selective tissue coagulation during thermally mediated procedures by using cryogen spray cooling. Various clinical procedures, such as laser treatment of hemangiomas, could potentially benefit from cryogen-spray cooling in conjunction with heating of tissues in order to confine tissue necrosis to subsurface regions.

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