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

Journal
Applied Optics, 35(19)

ISSN
0003-6935

Authors
Anvari, Bahman
Tanenbaum, B Samuel
Milner, Thomas E
et al.

Publication Date
1996-07-01

DOI
10.1364/ao.35.003314

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

Bahman Anvari, B. Samuel Tanenbaum, Thomas E. Milner, Kimberly Tang, Lih-Huei 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-damage confinement.

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, hemangiomas, and telangiectasias is based on the photocoagulation 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 thermal 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: superficial 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 tetrafluoroethane just prior to pulsed-laser irradiation can selectively cool the epidermis and yet permit photocoagulation of dilated port-wine stain blood vessels.[12,13] Successful blanching of port-wine strain 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/cm^2).[10,11,13]

B. Anavari, T. E. Milner, L.-H. Liaw, and J. S. Nelson are with the Beckman Laser Institute and Medical Clinic, University of California, Irvine, Irvine, California 92715; B. S. Tannenbaum and K. Kalafus are with the Department of Engineering, Harvey Mudd College, Claremont, California 91711; K. S. Tang is with the Department of Dermatology, University of California, Irvine, Irvine, California 92715; and S. Kimel is with the Department of Chemistry, Technion–Israel Institute of Technology, Haifa, Israel. B. Anavari is also with the Department of Engineering, Harvey Mudd College, Claremont, California 91711.

Received 9 October 1995; revised manuscript received 11 January 1996.

0003-6935/96/193314-07$10.00/0

In this paper we present the results of an experiment using continuous Nd:YAG laser irradiation and cryogen spray cooling. The Nd:YAG laser ($\lambda = 1064$ nm) light is poorly absorbed by biological tissues and penetrates several millimeters into tissues to achieve deep photocoagulation, 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 (LaserSonics Inc., Santa Clara, Calif.) was used as a heat source to induce deep tissue photocoagulation in freshly excised rabbit liver. Although liver is a relatively homogeneous medium (unlike skin), it nevertheless serves as a convenient tissue phantom in which photocoagulation 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 core-diameter silica multimode optical fiber was 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.

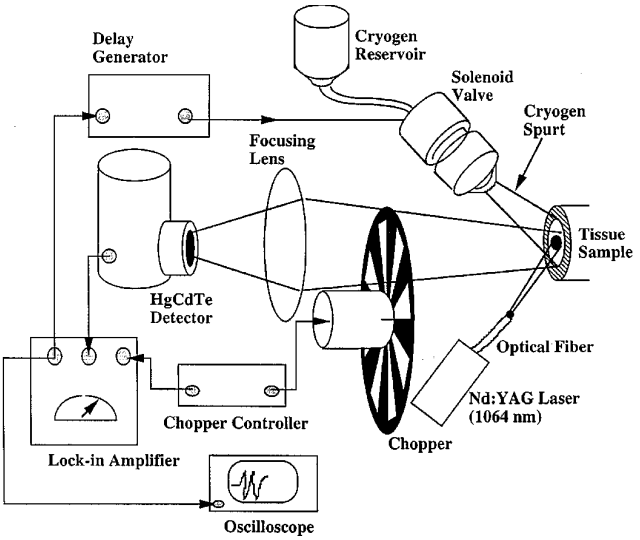


Fig. 1. Schematic diagram of the experimental apparatus for cryogen spray cooling and Nd:YAG laser irradiation measurement of surface temperature during the experiment is made by IR radiometry.

Table 1. Laser-Irradiation Parameters

Parameter Set	Delivered Laser Power (W)	Irradiation Time t_{irrad} (s)	Laser-Irradiated Spot Diameter (mm)	Irradiance ϕ_0 (kW/m^2)
1	5, 10	120, 90	7	130, 260
2	20	10, 15, 20	7	520
3	20	10	3	2,830
4	40, 50	30, 15	9	630, 790

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-mm^2 liquid-N_2-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 $f/1$ 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 SR850, 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 ($\epsilon \approx 0.97$) 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 FerroTec, 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

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, $t_{irrad} = 15$ s). Photocoagulated tissue (≈4 mm thick, 5-mm maximum width) below a protected layer (≈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, $t_{irrad} = 10$ 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/m^2) that the amount of heat generated was greater than that which could be removed by cooling.

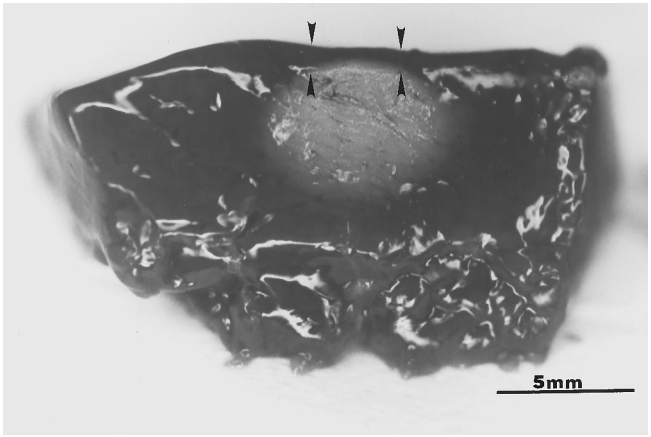
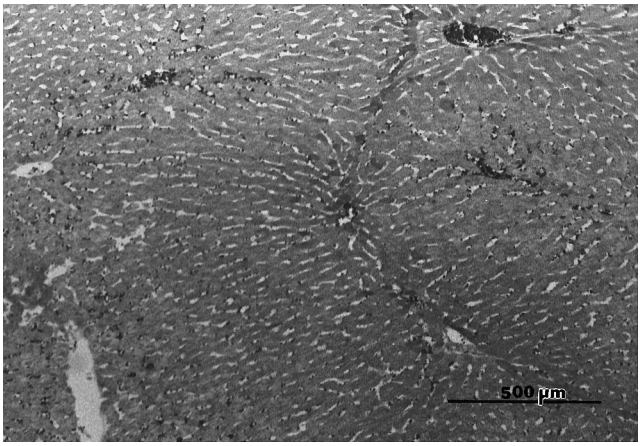
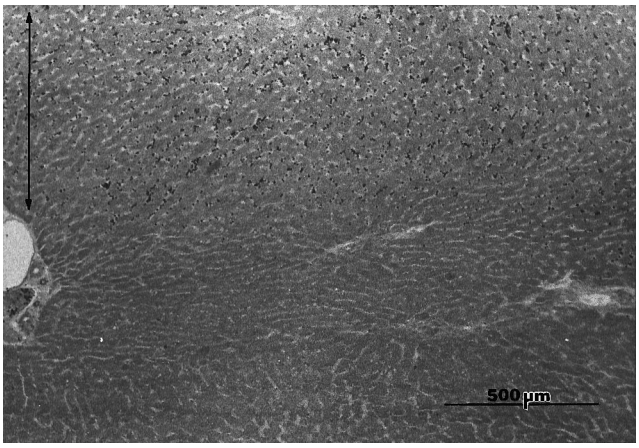


Fig. 2. Gross cross section of rabbit liver tissue irradiated with Nd:YAG laser (parameter set 2, $t_{irrad} = 15$ s) repetitively cooled with 50-ms chlorodifluoromethane spurts. The protected region is indicated by the region between the arrows. The surface of the tissue is not visible in the figure.



(a)



(b)

Fig. 3. Histologic sections of (a) nonirradiated, and (b) irradiated tissue (parameters set 2, $t_{irrad} = 10$ s) while repetitively cooled with 50-ms chlorodifluoromethane spurts.

An example of the recorded radiometric surface temperature in response to laser irradiation (parameter set 2, $t_{irrad} = 15$ 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

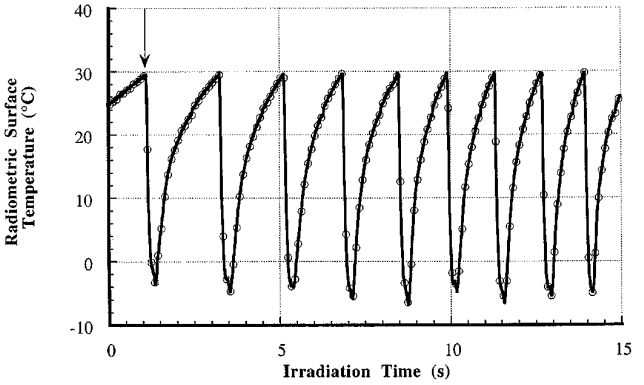


Fig. 4. Radiometric surface temperature of liver tissue during Nd:YAG laser irradiation (parameter set 2, $t_{irrad} = 15$ s) and repetitive spray cooling with 50-ms chlorodifluoromethane spurts. The arrow indicates a time at which a cryogen spurt was released.

heat-conduction equation. In one dimension,

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

where T (°C) is the temperature, z (m) is the distance into the tissue (with the origin at the tissue surface), t(s) is time, α is the thermal diffusivity (2×10^{-7} m^2/s for liver tissue[15]), k is the tissue thermal conductivity (0.59 W/mK),[15] and Q_L (W/m^3) is the volumetric heat generation caused by laser irradiation. We assume that Q_L is distributed as

$$Q_L(z) = \mu_a \phi_0 \exp(-\mu_a z), \tag{2}$$

where μ_a is the absorption coefficient of liver at 1064 nm (0.88 mm^{-1}),[16] and ϕ_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.[17–20] Backscattering of laser light is expected to cause the maximum value of Q_L to occur just below the surface of the sample.[21] Deep in the tissue, Q_L is overestimated by the neglect of scattering.[22] 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.[23]

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

$$T(z = 0, t) = T_{thresh} - \left[\frac{T_{thresh} - T_{min}}{2}(1 - \cos 2\pi f t)\right]. \tag{3}$$

Here, T_{thresh} is the prespecified threshold temperature (30 °C), T_{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:

$$T(z,t) = \Delta T_L(z,t) + \Delta T_C(z,t) + T_{thresh}, \tag{4}$$

where[24]

$$\Delta T_L = \frac{\phi_0}{\mu_a k}\left\{\operatorname{erfc}(\tilde{z}) - \exp(-\mu_a z) + \frac{\exp(-\tilde{z}^2)}{2}\left[\operatorname{erfcx}(\tilde{\mu}_a - \tilde{z}) - \operatorname{erfcx}(\tilde{\mu}_a + \tilde{z})\right]\right\}, \tag{5}$$

$$\Delta T_C = \frac{T_{min} - T_{thresh}}{2}\left[\operatorname{erfc}(\tilde{z}) - \exp(-\beta z)\cos(2\pi f t - \beta z)\right], \tag{6}$$

with

$$\tilde{\mu}_a = \mu_a\sqrt{\alpha t}, \qquad \tilde{z} = z/2\sqrt{\alpha t}, \qquad \beta = \sqrt{\pi f/\alpha}, \tag{7}$$

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

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. 5(a)]. With the assumption that the threshold temperature for photocoagulation necrosis is approximately 55 °C when this temperature is maintained for seconds[25] [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 (≈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. 5(b)]. The thickness of photocoagulated tissue

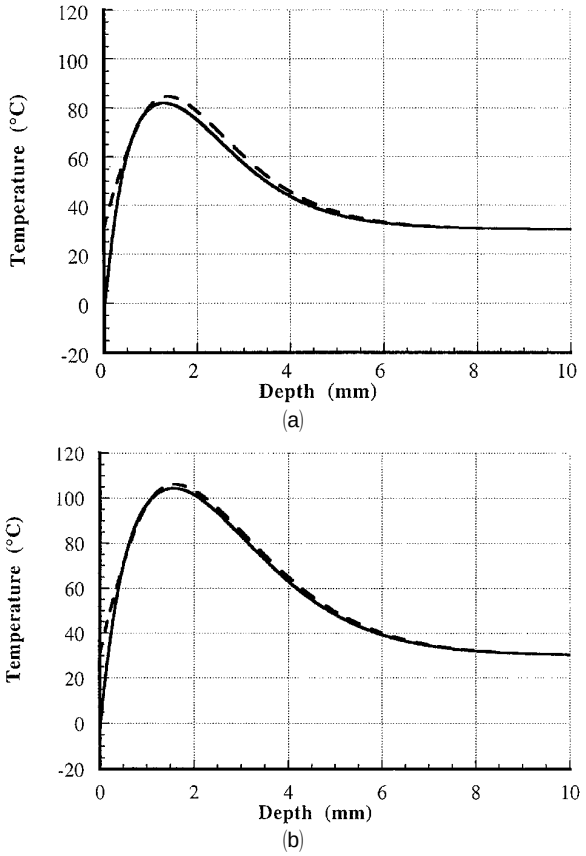


Fig. 5. Calculated temperature distributions in response to Nd:YAG laser irradiation (5 W, 7-mm irradiated spot diameter) and periodic cryogen spray cooling at 0.625 Hz. The solid curves correspond to minimum surface temperatures at (a) $t = 7.2$ s, and (b) $t = 15.2$ s; the dashed curves correspond to maximum surface temperatures at (a) $t = 8.0$ s, and (b) $t = 16.0$ s.

has increased, while the temperature of the superficial tissue still remains below 55 °C.

The heat flux q ($\mathrm{W/m^2}$), removed at the surface is obtained by the superposition of fluxes arising from cooling and laser irradiation:

$$q(t) = q_L(t) + q_C(t), \tag{8}$$

where

$$q_L(t) = k \left.\frac{\partial \Delta T_L(z, t)}{\partial z}\right|_{z=0} = \phi_0[1 - \mathrm{erfcx}(\tilde{\mu}_a)], \tag{9}$$

$$q_C = k \left.\frac{\partial \Delta T_C(z, t)}{\partial z}\right|_{z=0} = k\left(\frac{T_{\min} - T_{\mathrm{thresh}}}{2}\right) \times \left[-\frac{1}{\sqrt{\pi \alpha t}} + \sqrt{2}\beta \cos\left(2\pi f t + \frac{\pi}{4}\right)\right]. \tag{10}$$

The calculated values of the heat flux removed at the tissue surface in response to laser irradiation (5 W, 7-mm irradiated spot diameter), $\mu_a = 0.88\ \mathrm{mm^{-1}}$, and cryogen spray cooling ($f = 0.625$) are shown in Fig. 6. The oscillatory behavior results from the

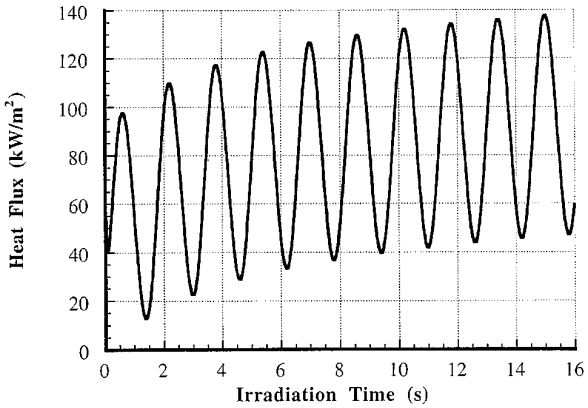


Fig. 6. Calculated values of the heat flux removed at the tissue surface in response to Nd:YAG laser irradiation (5 W, 7-mm irradiated spot diameter) and periodic cryogen-spray cooling at 0.625 Hz.

nature of boundary-condition equation (3). The minimum and maximum values increase with irradiation time and approach steady-state values.

The calculated thicknesses of the photocoagulated and protected tissues as functions of the irradiation time for three different laser powers (5, 10, and 20 W) but constant delivered laser energies (80 J) and irradiated spot diameters (7 mm) are shown in Fig. 7. Calculations of the thickness of photocoagulated tissue were based on the assumption of 55 °C as the threshold temperature for tissue whitening. The physically unrealistic ripples in the curves result from the oscillatory boundary condition. The delay time before tissue photocoagulation begins increases as the laser power is lowered [Fig. 7(a)]. The time required to photocoagulate a given tissue thickness is inversely related to the laser power.

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 ϕ_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-

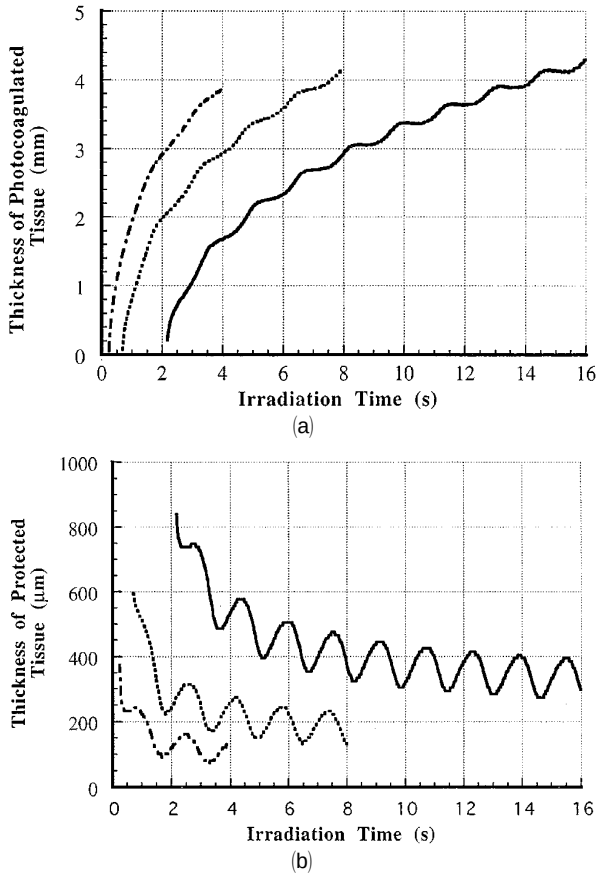


Fig. 7. Calculated thicknesses of (a) photocoagulated, and (b) protected regions in response to Nd:YAG laser irradiation at 5 W for 16 s (solid curve), 10 W for 8 s (dotted curve), and 20 W for 4 s (dotted–dashed curve) with periodic cryogen spray cooling at 0.625 Hz. The delivered laser energy and irradiated spot diameter are constant at 80 J and 7 mm, respectively.

turation[27] and requires a more accurate value for the tissue absorption coefficient.[28]

As illustrated with a liver tissue phantom and by our theoretical analysis, the feasibility of inducing spatially selective coagulation with cryogen spray cooling may have broad clinical implications. For example, successful laser treatment of large or bulky hemangiomas is based on photocoagulating blood vessels while avoiding thermal injury to the epidermis and papillary dermis. Because of the relatively deep tissue penetration at 1064 nm, the Nd:YAG laser has been used to coagulate bulky hemangiomas[29,30] in which the dermis is almost completely replaced by blood vessels.[31] Nevertheless thermal injury to the epidermis and papillary dermis remains a major concern.[5] The repetitive application of cryogen spray cooling during continuous Nd:YAG laser irradiation may provide a method with which the epidermis and papillary dermis can be protected.

Cooling tissue with water or air during microwave heating and continuous laser irradiation has been shown to be effective for protecting superficial tissues.[19,32,33] Cryogen spray cooling can offer comparable 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 ~16 °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 spatiall 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.

This work was supported by grants from the Institute of Arthritis and Musculoskeletal and Skin Disease (1R29-AR41638-01A1, R15-AR43403-01, and 1R01-AR42437-01A1 at the National Institutes of Health, the Whitaker Foundation (Special Opportunity Grant), the Biomedical Research Technology Program (R03-RR0698), the Dermatology Foundation, the Department of Energy (DE-F6-3-91ER6122), the Office of Naval Research (N0014-91-0134), the National Institutes of Health (RR01192), the Society of Photo-Optical and Instrumentation Engineers (Educational Grant in Optical Engineering), and the Beckman Laser Institute Endowment. Assistance from Binh Nguyen and Julie Kim during experimental procedures and histologic preparation is greatly appreciated.

References

1. M. A. Astrahan, K. Imanaka, G. Jozsef, F. Ameye, L. Baert, M. D. Sapozink, S. Boyd, and Z. Petrovich, "Heating characteristics of a helical microwave applicator for transurethral hyperthermia of benign prostatic hyperplasia," Int. J. Hyperthermia. **7,** 141–155 (1991).
2. B. M. Achauer, V. M. Vander Kam, and M. Berns, *Lasers in Plastic Surgery and Dermatology* (Thieme, New York, 1992).
3. A. J. Welch and M. J. C. van Gemert, *Optical–Thermal Response of Laser-Irradiated Tissue* (Plenum, New York, 1995).
4. R. R. Anderson and J. A. Parrish, "Selective photothermolysis: precise microsurgery by selective absorption of pulsed radiation," Science **220,** 524–527 (1983).
5. B. M. Achauer and V. M. Vander Kam, "Capillary hemangioma (strawberry mark) of infancy: comparison of argon and Nd:YAG laser treatment," Plast. Reconstr. Surg. **84,** 60–70 (1988).
6. J. S. Nelson and J. Applebaum, "Clinical management of port-wine stain in infants and young children using the flashlamp-pulsed dye laser," Pediatrics **29,** 503–508 (1990).
7. R. Ashinoff and R. G. Geronemus, "Capillary hemangiomas

and treatment with the flashlamp-pulsed dye laser," Arch. Dermatol. **127,** 202–205 (1991).
8. J. A. Werner, B. M. Lippert, P. Hoffmann, and H. Rudert, "Nd:YAG laser therapy of voluminous hemangiomas and vascular malformations," Adv. Oto-Rhono-Laryngol. **49,** 75–80 (1995).
9. B. Anvari, T. E. Milner, B. S. Tanenbaum, S. Kimel, L. O. Svaasand, and J. S. Nelson, "Selective cooling of biological tissues: application for thermally mediated therapeutic procedures," Phys. Med. Biol. **40,** 241–252 (1995).
10. B. Anvari, T. E. Milner, B. S. Tanenbaum, S. Kimel, L. O. Svaasand, and J. S. Nelson, "Dynamic epidermal cooling in conjunction with laser treatment of port wine stains: theoretical and preliminary clinical evaluations," Lasers Med. Sci. **10,** 105–112 (1995).
11. J. S. Nelson, T. E. Milner, B. Anvari, B. S. Tanenbaum, S. Kimel, L. O. Svaasand, and S. L. Jacques, "Dynamic epidermal cooling during pulsed laser treatment of port wine stain—a new methodology with preliminary clinical evaluation," Arch. Dermatol. **131,** 695–700 (1995).
12. B. Anvari, B. S. Tanenbaum, T. E. Milner, S. Kimel, L. O. Svaasand, and J. S. Nelson, "A theoretical study of the thermal response of skin to cryogen spray cooling and pulsed laser irradiation: implications for treatment of port wine stain birthmarks," Phys. Med. Biol. **40,** 1451–1465 (1995).
13. J. S. Nelson, T. E. Milner, B. Anvari, B. S. Tanenbaum, L. O. Svaasand, and S. Kimel, "Dynamic epidermal cooling in conjunction with laser-induced photothermolysis of port wine stain blood vessels," Lasers Surg. Med. (to be published).
14. G. M. Hale and M. R. Querry, "Optical constants of water in the 200 nm to 200 μm wavelength," Appl. Opt. **12,** 555–563 (1973).
15. F. A. Duck, *Physical Properties of Tissue. A Comprehensive Reference Book* (Academic, London, 1990).
16. J. W. Pickering, P. Posthumus, and M. J. C. van Gemert, "Continuous measurement of the heat-induced changes in the optical properties (at 1,064 nm) of rat liver," Lasers Surg. Med. **15,** 200–205 (1994).
17. G. Yoon, A. J. Welch, M. Motamedi, and M. C. J. van Gemert, "Development and application of three-dimensional light distribution model for laser irradiated tissue," IEEE J. Quantum Electron. **23,** 1721–1732 (1987).
18. L. Wang and S. L. Jacques, "Hybrid model of Monte Carlo simulation and diffusion theory for light reflectance by turbid media," J. Opt. Soc. Am. A **10,** 1746–1752 (1993).
19. B. Anvari, S. Rastegar, and M. Motamedi, "Modeling of intraluminal heating of biological tissue: implications for treatment of benign prostatic hyperplasia," IEEE Trans. Biomed. Eng. **41,** 854–864 (1994).
20. D. J. Smithies and P. H. Butler, "Modeling the distribution of laser light in port-wine stains with the Monte Carlo method," Phys. Med. Biol. **40,** 701–731 (1995).
21. R. R. Anderson, H. Beck, U. Bruggemann, W. Farinelli, S. L. Jacques, and J. A. Parrish, "Pulsed photothermal radiometry in turbid media: internal reflection of backscattered radiation strongly influences optical dosimetry," Appl. Opt. **28,** 2256–2262 (1989).
22. S. Rastegar, M. Motamedi, A. J. Welch, and L. J. Hayes, "A theoretical study of the effect of optical properties in laser ablation of tissue," IEEE Trans. Biomed. Eng. **36,** 1180–1187 (1989).
23. L. O. Svaasand, "On the propagation of thermal waves in blood perfused tissues," Lasers Life Sci. **2,** 289–311 (1988).
24. H. S. Carslaw and J. C. Jaeger, *Conduction of Heat in Solids,* 2nd ed. (Clarendon, Oxford, UK, 1959), pp. 62 and 79.
25. M. J. C. van Gemert, A. J. Welch, I. D. Miller, and O. T. Tan, "Can physical modeling lead to an optimal laser treatment of port-wine stains?" in *Laser Applications in Medicine and Biology,* M. L. Wolbarsht, ed. (Plenum, New York, 1991), Vol. 5, pp. 199–275.
26. B. Anvari, M. Motamedi, J. H. Torres, S. Rastegar, and E. Orihuela, "Effects of surface irrigation on the thermal response of tissue during laser irradiation," Lasers Surg. Med. **14,** 386–395 (1994).
27. M.-S. Si, T. E. Milner, B. Anvari, and J. S. Nelson, "Dynamic heat capacity changes of laser irradiated type I collagen films," Lasers Surg. Med. **19,** 17–22 (1996).
28. J. H. Torres, A. J. Welch, I. I. Çilesiz, and M. Motamedi, "Tissue optical property measurements: overestimation of absorption coefficient with spectrophotometric techniques," Lasers Surg. Med. **14,** 249–257 (1994).
29. H. Rosenfeld and R. Sherman, "Treatment of cutaneous and deep vascular lesions with the Nd:YAG laser," Lasers Surg. Med. **6,** 20–24 (1986).
30. D. B. Apfelberg, T. Smith, H. Lash, D. N. White, and M. R. Maser, "Preliminary report on use of the neodymium-YAG laser in plastic surgery," Lasers Surg. Med. **7,** 189–198 (1987).
31. J. B. Mulliken and A. E. Young, *Vascular Birthmarks* (Saunders, Philadelphia, Penn., 1988).
32. M. M. Yeh, B. S. Trembly, E. V. Douple, T. P. Ryan, P. J. Hoopes, E. Jonsson, and J. A. Heaney, "Theoretical and experimental analysis of air cooling for intracavitray microwave hyperthermia applicators," IEEE Trans. Biomed. Eng. **41,** 874–882 (1994).
33. P. S. Debicki, M. Okoniewski, E. Okoniewska, P. N. Shrivastave, A. M. Debicka, L. V. Baert, and Z. Petrovich, "Cooled microwave transrectal applicator with adjustable directional beam for prostate treatment," Int. J. Hypertherm. **11,** 95–108 (1995).