

Comparative study of cryogen spray cooling with R-134a and R-404a: implications for laser treatment of dark human skin

Tianhong Dai
Mohammad A. Yaseen
Parmeswaran Diagaradjane
Rice University
Department of Bioengineering
MS 142, P.O. Box 1892
Houston, Texas 77251

David W. Chang
University of Texas M.D. Anderson Cancer Center
Department of Plastic Surgery
Houston, Texas 77030

Bahman Anvari
Rice University
Department of Bioengineering
MS 142, P.O. Box 1892
Houston, Texas 77251

Abstract. Cutaneous laser treatment in dark skin patients is challenging due to significant light absorption by the melanin at the basal layer of epidermis, which can result in irreversible nonspecific thermal injury to the epidermis. Cryogen spray cooling (CSC) with R-134a (boiling point $\approx -26.2^\circ\text{C}$ at 1 atm), which is currently used during cutaneous laser treatment, has shown poor efficacy in protecting dark human skin. We investigated the potential of CSC with R-404a (boiling point $\approx -46.5^\circ\text{C}$ at 1 atm), which has a lower boiling point than R-134a, for improved therapeutic outcome in dark human skin at three levels: *in vitro* (epoxy resin skin phantom), *ex vivo* (normal dark human skin sample), and *in vivo* (skin of the rabbit external ear). The skin phantom was used to acquire the surface and internal temperature profiles in response to CSC with R-134a or R-404a at various spurt durations, based upon which CSC-induced heat removal from the skin phantom was estimated using an algorithm that solved a one-dimensional inverse heat conduction problem. CSC with R-404a increased the temperature reductions within the phantom and subsequently the amount of heat removal from the phantom in comparison to that with R-134a. Normal *ex vivo* Fitzpatrick types V-VI human skin samples were used to investigate the thermal response of dark human skin epidermis to CSC (R-134a or R-404a) at various spurt durations in conjunction with 595-nm pulsed dye laser irradiation at various radiant exposures. Cryogen R-404a increased the threshold radiant exposures for irreversible thermal injury to the epidermis in dark pigmentation skin. No obvious CSC-induced morphological changes to human skin was observed when sprayed with R404-a spurts using durations up to 300 ms. *In vivo* rabbit ear vasculature was used as a model of cutaneous anomalies to assess the influences of CSC (with R-134a or R-404a) on the photothermolysis of dermal blood vessels. CSC (R-134a or R-404a) with the spurt durations of 100 to 300 ms increased the most superficial depth of thermally damaged dermal blood vessel compared with the sites without CSC, implying possible nonspecific cooling of superficial dermal blood vessels by the cryogen spurts with the settings applied. © 2006 Society of Photo-Optical Instrumentation Engineers. [DOI: 10.1117/1.2338001]

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1 Introduction

The ideal clinical objective during laser treatment of cutaneous vascular anomalies such as port wine stains is to destroy subsurface targeted blood vessels while avoiding or minimizing nonspecific thermal injury to the overlying epidermis and other skin structures. Although pulsed dye laser irradiation (at a 585- or 595-nm wavelength) has proven to be a superior

approach for treatment of port wine stains, a large segment of the patient population, namely those with dark skin, cannot currently benefit from laser treatment. Nonspecific thermal injury to the epidermis is a frequent outcome in these patients.¹⁻⁶ The principal reason for the poor clinical results in these patients is their skin pigmentation level: a large fraction of the light that is intended to reach and destroy the targeted subsurface structures is absorbed by the melanin at the basal layer of epidermis.

Address all correspondence to Bahman Anvari, Bioengineering, Rice University, 6100 Main St. – MS-142, Houston, TX 77251-1892; Tel: 713-348-3019; Fax: 713-348-5877; E-mail: anvari@rice.edu

One technique to minimize or eliminate laser-induced non-specific thermal injury to the epidermis is to selectively cool the superficial layers of skin. A technology based on spraying a short cryogen spurt (on the order of milliseconds) onto skin surface immediately prior to laser irradiation has been reported.^{7,8} The cryogen spurt duration is sufficiently short so that only the superficial layers of skin are selectively cooled while the targeted subsurface structures are not affected. Cryogen R-134a (boiling point $\approx -26.2^\circ\text{C}$ at 1 atm) is currently the cooling agent used in clinical settings.⁹⁻¹³ Recent studies^{12,14,15} have demonstrated that thermal injury to the epidermis could be successfully prevented in lightly to moderately pigmented human skin (types I-IV, according to Fitzpatrick classification¹⁶) when applying 100 to 300 ms R-134a spurts. However, in darkly pigmented human skin (types V-VI), skin epidermis could not survive from nonspecific thermal injury even when irradiated at very low radiant exposures (e.g., $D_0=4\text{ J/cm}^2$ for type VI skin) in conjunction with R-134a spurts.^{11,14,15} Long-term side effects such as skin texture change and hypopigmentation were noted when treating dark skin patients.¹¹

The reason for the poor efficacy of cryogen spray cooling (CSC) with R-134a in protecting dark human skin from non-specific thermal injury is the insufficient CSC-induced heat removal from skin epidermis to counteract laser-induced heat generation in skin epidermis. Cryogen R-404a, which possesses a lower boiling point (boiling point $\approx -46.5^\circ\text{C}$ at 1 atm) than R-134a, is expected to increase the heat removal from the skin epidermis given the same spurt duration. In this study, we investigated the potential of CSC with R-404a for improved therapeutic outcome in dark human skin at three levels: *in vitro*, *ex vivo*, and *in vivo*. We used an *in vitro* model (a skin phantom made of epoxy resin) with an embedded thermocouple to estimate the CSC-induced temperature deductions (or heat removal) in response to R-134a or R-404a spurts. Using *ex vivo* normal dark human skin samples, we investigated the threshold radiant exposures for irreversible thermal injury to the epidermis in response to CSC with R-134a or R-404a. Finally, we used *in vivo* rabbit ear vasculature to assess the influence of CSC with R-134a or R-404a on the photothermolysis of dermal blood vessels.

2 Materials and Methods

2.1 Laser, Cryogen, and CSC Delivery Device

The flashlamp-pumped pulsed dye laser Candela Sclero-Plus™ (Candela Inc., Wayland, MA) was used to irradiate the *ex vivo* normal dark human skin samples and *in vivo* rabbit external ears. This laser provided user-specified discrete radiant exposures between 4 to 15 J/cm². The wavelength was tuned to 585 or 595 nm. The pulse duration was fixed at 1.5 ms, spot size 7 mm.

The cryogen types used in the study were R134-a and R-404a (Dupont Corp., Wilmington, DE). While there are various refrigerants (Table 1) and we have previously reported on the use of R-407c and R-22,¹⁷⁻²⁰ we chose to compare R-134a (currently used in clinical setting) with R-404a based on the following criteria: (1) environmental compatibility, (2) a sufficiently low boiling point but not inducing cryoinjury in the absence of laser irradiation, and (3) practical delivery (e.g., our previous attempts at delivering frozen CO₂ pro-

Table 1 Examples of refrigerants and their boiling points at atmospheric pressure.

Refrigerant	Boiling Points ($^\circ\text{C}$) at Atmospheric Pressure
R-134a ^a	-26
R-22	-42
R-407c ^a	-43
R-404a ^a	-48
Dry Ice ^a (frozen CO ₂)	-78

^aDenotes an environmentally compatible refrigerant.

duced an intense sound, similar to gun shot, when injected through a valve). Based on these criteria, R-22 and dry ice did not serve as candidate materials. R-407c would have been a reasonable choice; however, it has a slightly warmer boiling point than R-404a.

Cryogen R-134a (1,1,1,2-tetrafluoroethane) is environmentally compatible, nontoxic with its boiling point of -26.2°C at atmospheric pressure, and has been approved by the Food and Drug Administration for use in cutaneous laser treatment. The composition of the R-404a as a percentage of weight is 52% trifluoroethane (R-143a), 44% pentafluoroethane (R-125), and 4% 1,1,1,2-tetrafluoroethane (R-134a). The boiling point of R-404a is -46.5°C at atmospheric pressure. Cryogen R-404a is also environmentally compatible and classified as a high-pressure refrigerant used in commercial applications of low temperature cooling systems.²¹

A commercial fuel injector (Standard Motor Products, 800-1257N, Long Island City, NY) was used to deliver R-134a or R-404a. A nozzle with a 1-mm diameter orifice was attached to the injector to produce a uniform spray cone. The injector was aimed toward the sprayed surface at an angle of 30 deg with respect to the normal. The injector-to-surface distance was 85 mm for all experiments, which was the optimized spray distance for the maximum temperature reduction within a skin phantom for this particular cryogen delivery device.²² A previous study demonstrated that various cryogen delivery devices produce different amounts of Q [see Eq. (1)].²² The "optimum" injector-to-surface distance is, therefore, device dependent. In all experiments, the cryogen spurt duration as well as the time delay between the termination of cryogen spurt and the onset of laser pulse (in *ex vivo* and *in vivo* studies) were controlled by using a programmable digital delay generator (DG 535, Stanford Research System, Sunnyvale, CA).

2.2 Skin Phantom

A skin phantom made of epoxy resin (EP30, Master Bond Inc., Hackensack, NJ) with an embedded 30- μm diameter type *K* thermocouple (Chromega®-Alomega®) (Omega Engineering, Inc., Stamford, CT) positioned at a 100- μm ($\pm 5\ \mu\text{m}$) depth below the phantom surface was used to acquire the temperature profiles in response to CSC. The thermal diffusivity of the phantom was $0.843 \times 10^{-7}\text{ m}^2\text{ s}^{-1}$, which is within the range of human skin ($6.9 \times 10^{-8}\text{ m}^2\text{ s}^{-1}$ to

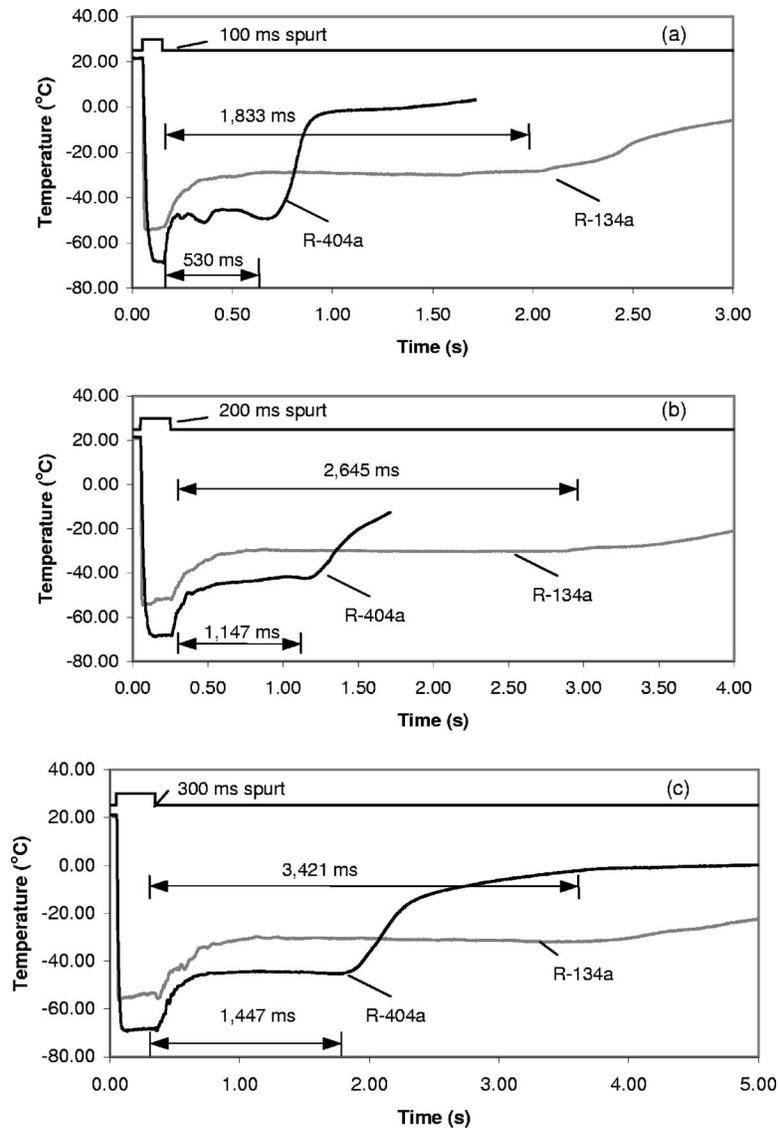


Fig. 1 Comparison of cryogen film temperature on the surface of the skin phantom in response to R-404a and R-134a spurts: (a) $\tau_{\text{CSC}}=100$ ms, (b) $\tau_{\text{CSC}}=200$ ms, (c) $\tau_{\text{CSC}}=300$ ms.

$1.1 \times 10^{-7} \text{ m}^2 \text{ s}^{-1}$).²³ Such a phantom has also been used by other investigators^{24–32} to investigate the effects of cryogen droplet size, spray density, droplet velocity, diameter, and film height on the CSC-induced heat removal from the phantom. Good agreements between computed temperature profiles and those measured in an epoxy resin phantom have been obtained,^{24,26} indicating the effectiveness of this *in vitro* model. In addition, another type *K* thermocouple with a 60- μm diameter sensing bead was used to measure the cryogen film temperature on the surface of the skin phantom. Both thermocouples were connected to an InstruNet digital data acquisition system (Omega Engineering, Inc., Stamford, CT). The data acquisition rate during the temperature measurements was 1000 Hz.

The skin phantom, initially at room temperature ($\approx 21^\circ\text{C}$), was sprayed with R-134a or R-404a spurts at the durations of $\tau_{\text{CSC}}=100$, 200, and 300 ms. All temperature measurements were performed in triplicate for each setting.

Measured temperature profiles were subsequently used to estimate CSC-induced heat removal from skin phantom using an algorithm that solved an inverse heat conduction problem.²⁶ Additional details about the performance and accuracy of the algorithm can be found in Ref. 22.

2.3 *Ex Vivo* Dark Human Skin Sample

Normal *ex vivo* human skin samples of Fitzpatrick types V-VI were obtained from the abdomens of seven consenting adult females undergoing the transverse rectus abdominis myocutaneous (TRAM) flap procedures in the department of plastic surgery at the University of Texas M.D. Anderson Cancer Center (MDACC). The TRAM procedure extracts a skin and muscle section from the lower part of the abdomen for breast reconstruction. The redundant skin sections from TRAM procedure were then used for the study. The protocol to obtain the skin samples was approved by the Institutional Review Board at MDACC and the Rice University. The skin samples

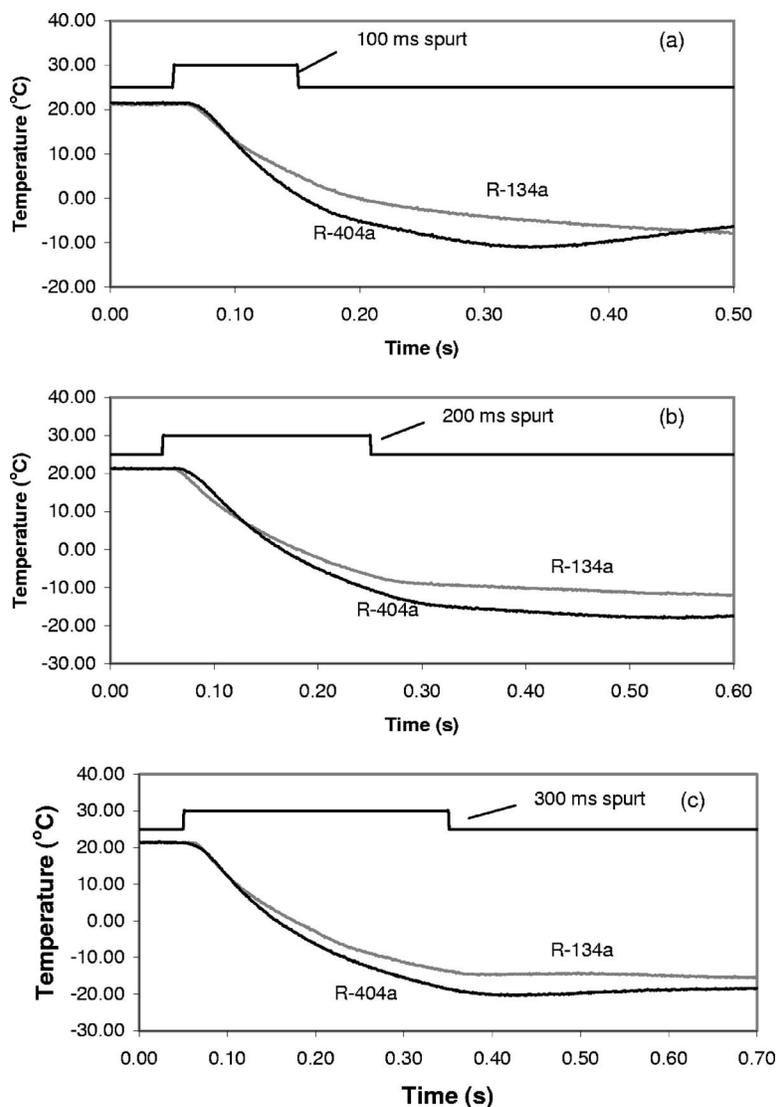


Fig. 2 Comparison of internal temperature at the depth of 100 μm below the surface of the skin phantom in response to R-404a and R-134a spurts: (a) $\tau_{\text{CSC}}=100$ ms, (b) $\tau_{\text{CSC}}=200$ ms, (c) $\tau_{\text{CSC}}=300$ ms.

were harvested with epidermis, dermis, and subcutaneous fat as a whole. Once the skin samples were harvested, they were stored in biohazard plastic bags to prevent the loss of water content and then delivered to the laboratory. Temperatures of the skin samples were approximately 21 °C (room temperature) before experimentation. Each *ex vivo* human skin sample was irradiated by 595-nm wavelength at the radiant exposures of $D_{0.595}=4, 6, 8, 10, 13,$ and 15 J/cm^2 in conjunction with CSC (R-134a or R-404a) at the spurt durations of $\tau_{\text{CSC}}=100, 200,$ and 300 ms, respectively. Based on the parameters used in clinic settings,¹³ the time delay between the termination of cryogen spurt and the onset of laser pulse was 20 ms throughout the *ex vivo* experiments. This time delay is believed to be sufficient to ensure a relatively calm cryogen film to form on the sprayed surface, but sufficiently short to allow for the majority of heat removal to occur during the spurt duration.³³ Biopsy specimens were taken from the irradiated sites by 6-mm punches, fixed in 10% buffered formalin, processed for histological sectioning, and stained with hematoxylin and

eosin (H&E). Thermal injury to the epidermis was evaluated by H&E histological observations.

2.4 *In Vivo* Rabbit External Ear

Two New Zealand Albino white rabbits were used for the *in vivo* study as a model for cutaneous anomalies because the skin on a rabbit's external ear contains plentiful dermal vasculature. It was observed from the histological sections of nonirradiated control sites that rabbit ears contained vasculature ranging from capillaries with diameters $<10 \mu\text{m}$ to blood vessels with a diameter of $250 \mu\text{m}$. The thicknesses of the rabbit ears ranged from 920 to 1190 μm . The protocol to carry out the animal experiments was approved by the Institutional Animal Care and Use Committee at the Rice University.

The animals were fed with standard laboratory diet and water *ad libitum*. At the time of the experiments, the rabbits weighed between 2.5 and 3.0 kg. Prior to laser irradiation,

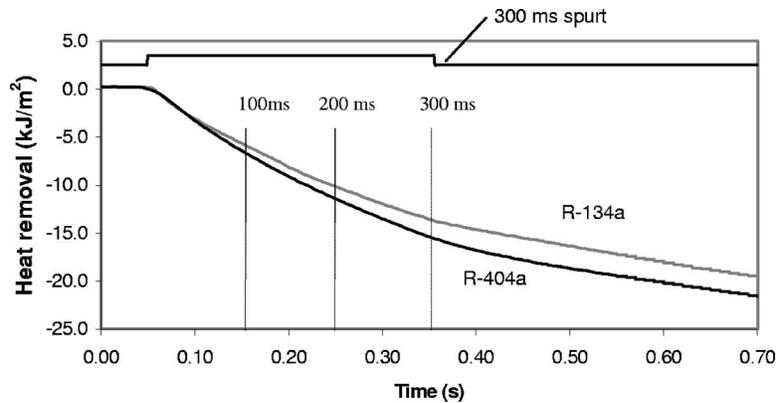


Fig. 3 Comparison of CSC-induced heat removal from the skin phantom in response to R-404a and R-134a spurts: (a) $\tau_{\text{CSC}}=100$ ms, (b) $\tau_{\text{CSC}}=200$ ms, (c) $\tau_{\text{CSC}}=300$ ms.

animals were anesthetized with 3% isoflurane in a lucite chamber until the animals reached the unconscious state. Once unconscious, the animals were maintained under anesthesia with 2% isoflurane delivered through a face mask. The animals were irradiated by 585-nm wavelength at two radiant exposures of $D_{0,585}=10$ and 13.5 J/cm² in conjunction with CSC (R-134a or R-404a) at the spurt durations of $\tau_{\text{CSC}}=100$, 200, and 300 ms, respectively. The 585-nm wavelength was used here because the blood absorption at 585 nm (≈ 191 cm⁻¹) is higher than that at 595 nm (≈ 43 cm⁻¹).³⁴ The time delay between the termination of cryogen spurt and the onset of laser pulse was also 20 ms throughout the *in vivo* experiments. One animal was sacrificed 2 h after laser irradiation and the other 4 days after irradiation. Biopsy specimens were taken from the irradiated sites by 6-mm punches, fixed in 10% buffered formalin, processed for histological sectioning, and stained with H&E. Thermal injury to the dermal vasculature was evaluated by H&E histological observations.

3 Results

3.1 Temperature Profiles and Heat Removal in Response to CSC With R-134a or R-404a

Figure 1 compares the averaged temperature measurements ($n=3$) of a cryogen film on the surface of a skin phantom in response to R-134a and R-404a spurts at the durations of 100, 200, and 300 ms. During cryogen spraying time, regardless of spurt duration, cryogen film temperature reached and remained almost constant at approximately -55°C for R-134a and -68°C for R-404a, well below the corresponding boiling points of the cryogens due to the evaporation of cryogen droplets during the flight from the injector to the skin phantom. Following cryogen spurt termination, liquid cryogen film remained on the phantom surface for a period of time (on the order of seconds), and the cryogen film temperatures during this period were near the boiling points of the cryogens: -26.2°C for R-134a and -46.5°C for R-404a. The lifetime of an after-spurt cryogen film was defined as the time between the cryogen spurt termination and the complete evaporation of the cryogen film. We quantified it from our temperature measurements using the 60- μm -bead type *K* thermocouple placed on the surface of a skin phantom. The complete evaporation time was identified as the time when the recorded tem-

perature began to increase above the respective boiling points for R-134a and R-404a. The lifetime of after-spurt cryogen film τ_a was directly related to the cryogen spurt duration τ_{CSC} . For the same spurt duration τ_{CSC} , the τ_a for R-404a was much shorter than that for R-134a. When τ_{CSC} was 100 ms, τ_a was approximately 530 ms for R-404a and 1833 ms for R-134a [Fig. 1(a)]. When τ_{CSC} was increased to 200 ms, τ_a were, respectively, 1147 and 2645 ms for R-404a and R-134a [Fig. 1(b)]. When τ_{CSC} was further increased to 300 ms, τ_a were 1447 and 3421 ms for R-404a and R-134a, respectively [Fig. 1(c)]. These differences may be due to the much lower boiling point of R-404a and subsequently the larger temperature difference between cryogen film and the surface temperature of

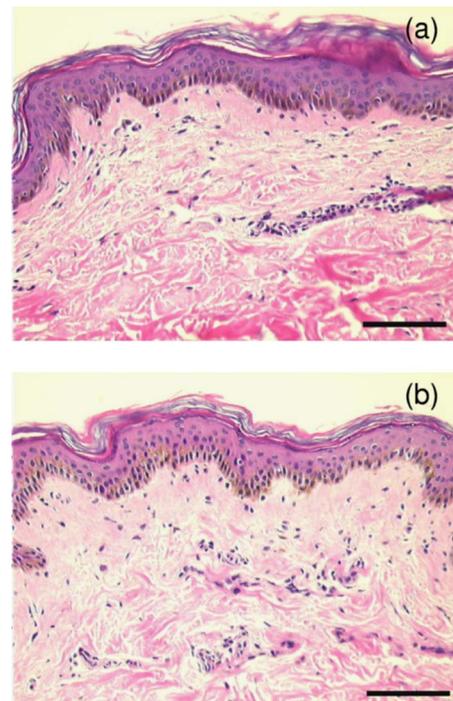


Fig. 4 H&E histological sections of (a) a control site (without CSC and laser irradiation) and (b) a site sprayed by a 300 ms R-404a spurt without laser irradiation from the same *ex vivo* human skin sample. Bars: 100 μm .

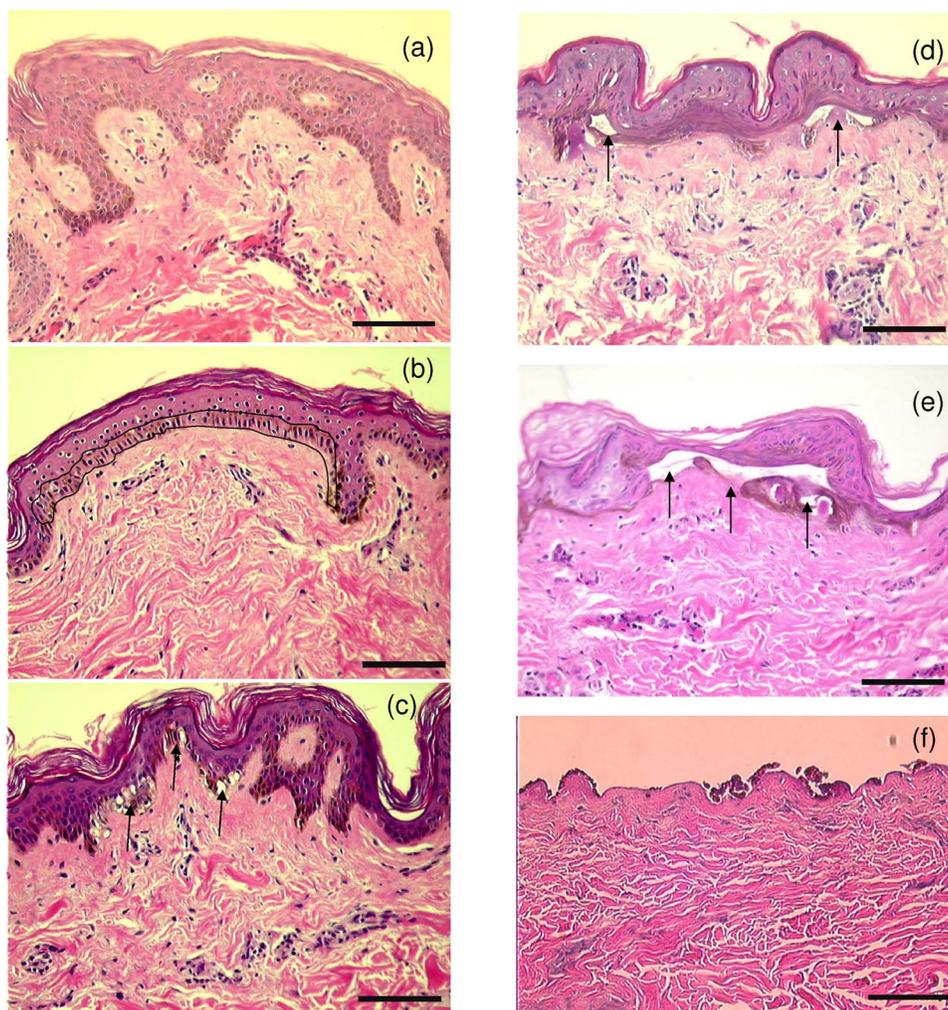


Fig. 5 Representative H&E histological sections showing various degrees of laser-induced thermal injury to the epidermis: (a) no damage, (b) basal cell elongation and hyperchromia (curves, $D_0=6$ J/cm², cryogen type R-404a, $\tau_{\text{CSC}}=300$ ms), (c) cytoplasmic vacuolization (arrows, $D_0=6$ J/cm², cryogen type R-404a, $\tau_{\text{CSC}}=100$ ms), (d) multiple basal lacunae formation (arrows, $D_0=10$ J/cm², cryogen type R-404a, $\tau_{\text{CSC}}=100$ ms), (e) partial basal layer separation (arrows, $D_0=13$ J/cm², cryogen type R-404a, $\tau_{\text{CSC}}=100$ ms), (f) complete basal layer separation ($D_0=15$ J/cm², cryogen type R-134a, $\tau_{\text{CSC}}=100$ ms). Bars: 100 μm .

the skin phantom, leading to a higher evaporation rate for R-404a film.

Figure 2 depicts the measured internal temperature profiles of skin phantom at the depth of approximately 100 μm below the phantom surface in response to R-134a or R-404a spurts at the spurt durations of 100, 200, and 300 ms. For $\tau_{\text{CSC}}=100$ ms, the temperature at the depth of 100 μm was 0.8 $^{\circ}\text{C}$ (temperature reduction $\Delta T=21-0.8=20.2^{\circ}\text{C}$) for R-404a and 5.2 $^{\circ}\text{C}$ ($\Delta T=15.8^{\circ}\text{C}$) for R-134a immediately after the spurt termination [Fig. 2(a)]. In response to $\tau_{\text{CSC}}=200$ ms, the corresponding temperatures were -10.6 $^{\circ}\text{C}$ ($\Delta T=31.6^{\circ}\text{C}$) for R-404a and -6.8 $^{\circ}\text{C}$ ($\Delta T=27.8^{\circ}\text{C}$) for R-134a immediately after the spurt termination [Fig. 2(b)]. With $\tau_{\text{CSC}}=300$ ms, the corresponding temperatures were -18.6 $^{\circ}\text{C}$ ($\Delta T=39.6^{\circ}\text{C}$) for R-404a and -13.7 $^{\circ}\text{C}$ ($\Delta T=34.7^{\circ}\text{C}$) for R-134a immediately after the spurt termination [Fig. 2(c)].

Using an algorithm that solved a one-dimensional inverse heat conduction problem,²⁶ we estimated the CSC-induced

heat removal from the skin phantom in response to R-404a or R-134a spurts at various spurt durations from the measured internal temperatures (Fig. 3). The heat removal profiles of R-404a and R-134a in response to 300-ms spurts suggest that a 100, 200, or 300-ms spurt of R-404a extracts, respectively, 12.1, 11.1, or 11.7% (6.4 versus 5.6 kJ/m², 11.5 versus 10.2 kJ/m², or 15.4 versus 13.6 kJ/m²) more heat than a R-134a spurt of equal duration. Therefore, the comparative increase in heat removal at the end of the cryogen spurt due to CSC with R-404 is approximately constant regardless of the spurt durations from 100 to 300 ms.

3.2 Histological Evaluation of Thermal Injury to the Epidermis

Figure 4 shows the H&E histological sections of a control site (without CSC and laser irradiation) and a site sprayed with a 300-ms R-404a spurt alone (without laser irradiation). The two sites were from the same skin sample. From the comparison of the two histological sections, it is observed that a

300-ms R-404a spurt did not induce obvious CSC-induced morphological changes within skin. Analysis of the experimental results of all *ex vivo* human skin samples was consistent with this finding.

The degrees of thermal injury to the epidermis were scored in the range of 0 to 5, corresponding to the range from the no visible thermal injury to maximum degree of thermal injury to the epidermis observed from H&E histological sections: 0—no visible thermal injury [Fig. 5(a)], 1—basal cell elongation and hyperchromia [Fig. 5(b)], 2—cytoplasmic vacuolization [Fig. 5(c)], 3—multiple basal lacunae formation [Fig. 5(d)], 4—partial basal layer separation [Fig. 5(e)], and 5—complete epidermal ablation [Fig. 5(f)].

Figure 6 shows the histological sections of two skin sites irradiated at the same laser irradiation parameters ($D_0 = 8 \text{ J/cm}^2$, $\tau_{\text{laser}} = 1.5 \text{ ms}$, $\lambda = 595 \text{ nm}$) but one sprayed with R-134a [Fig. 6(a)] and the other with R-404a [Fig. 6(b)]. Cryogen spray durations in both cases were 300 ms. Partial basal layer separation was observed on the site sprayed with R-134a [Fig. 6(a)], but no obvious thermal injury occurred on the site sprayed with R-404a [Fig. 6(b)].

Figure 7 presents the average threshold radiant exposures for irreversible thermal injury to the epidermis $D_{0,\text{thr}}$ with $D_{0,\text{thr}}$ defined as the minimum radiant exposure that induced a thermal injury to the epidermis with a score ≥ 2 . It was considered that an epidermal damage score of 2 could possibly lead to pigmentation change, and an epidermal damage score higher than 2 would induce cell necrosis.¹⁵ With $\tau_{\text{CSC}} = 100 \text{ ms}$, values of $D_{0,\text{thr}}$ were 4.8 J/cm^2 for R-134a, and 5.2 J/cm^2 for R-404a [Fig. 7(a)], but the difference was not statistically significant (one-tail t test $p = 0.3$). With $\tau_{\text{CSC}} = 200 \text{ ms}$, values of $D_{0,\text{thr}}$ were 4.8 and 6.8 J/cm^2 for R-134a and R-404a, respectively [Fig. 7(b)], and the difference was statistically significant (one-tail t test $p = 0.04$). With $\tau_{\text{CSC}} = 300 \text{ ms}$, values of $D_{0,\text{thr}}$ were 6.4 and 9.4 J/cm^2 , respectively, for R-134a and R-404a [Fig. 7(c)], and the difference was statistically significant (one-tail t -test $p = 0.04$).

3.3 Histological Evaluation of Thermal Injury to Dermal Blood Vessels

We assessed the effects of CSC on laser photothermolysis of dermal blood vessels using the *in vivo* skin on rabbit external ears. Figures 8(a)–8(c) are the H&E histological sections of the sites irradiated at the same irradiation parameters $D_0 = 13.5 \text{ J/cm}^2$, $\lambda = 585 \text{ nm}$, but the site in Fig. 8(a) was without CSC, while the sites in Figs. 8(b) and 8(c) were cooled with a 100-ms cryogen spurt of R-134a and R-404a, respectively. All sites were excised 2 h after the irradiation. Only the 2-h sites were selected here as the residual thermal injury in the 4-day sites was minimal and the differences between the 4-day sites were not distinguishable due to significant wound healing effects in rabbit ear vasculature.

It was found that CSC increased the depth at which the most superficial thermal injury to the blood vessel occurred (regardless of the degree of thermal injury) (ovals), implying that CSC applied at these parameters resulted in some cooling of superficial blood vessels. The depths of the most superficial thermal damage to dermal blood vessels for the sites shown in Figs. 8(a)–8(c) were approximately 70, 165, and 270 μm , respectively. Table 2 also indicates a general tendency that the

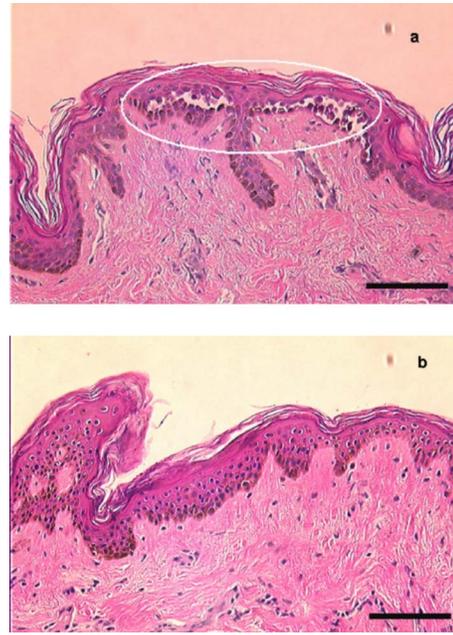


Fig. 6 H&E histological sections of two skin sites irradiated at the same laser irradiation parameters ($D_0 = 8 \text{ J/cm}^2$, $\tau_{\text{laser}} = 1.5 \text{ ms}$, $\lambda = 595 \text{ nm}$) but precooled with R-134a and R-404a at $\tau_{\text{CSC}} = 300 \text{ ms}$, respectively: (a) R-134a, (b) R-404a. Oval: Epidermal damage of partial basal layer separation. Bars: $100 \mu\text{m}$.

sites with cooling (sites 1 to 12) show deeper locations of the most superficial thermal injury to the blood vessel than those without cooling (sites 13 and 14). Fluctuations in the depths of the most superficial vascular damage in Table 2 with increased cryogen spurt durations were believed to be caused by the biological variability, e.g., the distributions of dermal blood vessels in different sites. There were no significant differences in the depths of the most superficial thermal injury to the blood vessel between the sites cooled by R-134a and those by R-404a (paired t test for comparing means,³⁵ $p = 0.96$), implying that using R-404a instead of R-134a may not interfere with the spatial selectivity of CSC.

4 Discussion

CSC-induced heat removal from a human skin surface during cryogen spraying time can be determined by³⁶

$$Q = \int_0^{\tau_{\text{CSC}}} h(T_{\text{skin},s} - T_{\text{film}}) dt \quad (1)$$

where Q is the CSC-induced heat removal per unit area from human skin surface (J/m^2), h is the heat transfer coefficient ($\text{W/m}^2\text{ }^\circ\text{C}$), T_{film} is cryogen film temperature ($^\circ\text{C}$), $T_{\text{skin},s}$ is skin surface temperature ($^\circ\text{C}$), τ_{CSC} is cryogen spurt duration (s), t is time (s). Based on Eq. (1), there are three means to enhance CSC-induced heat removal from human skin. The first is to increase the heat transfer coefficient h . This can be achieved, for example, by increasing the impinging droplet Weber number,^{32,37} which is defined as

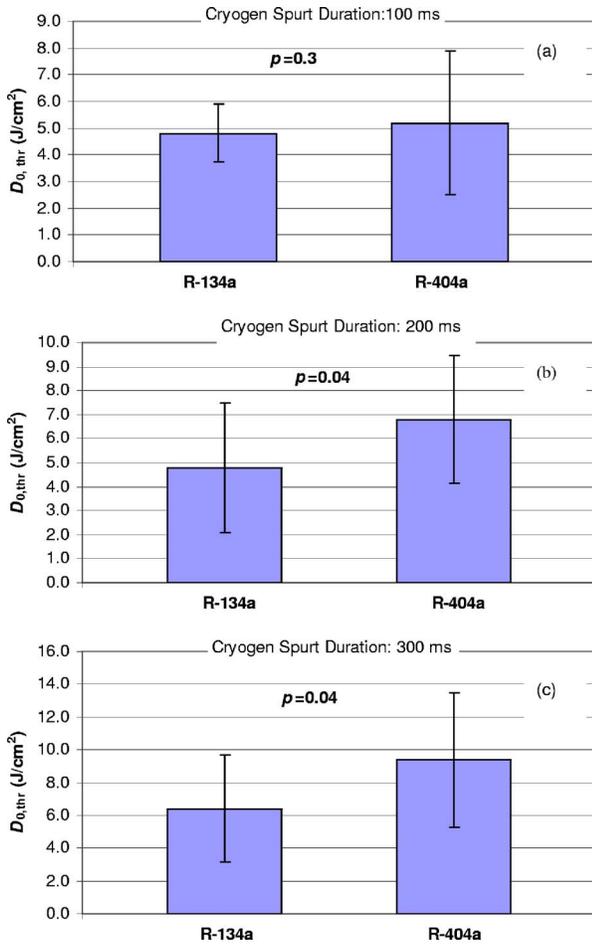


Fig. 7 Comparison of the threshold radiant exposures at 595-nm wavelength for irreversible thermal injury to the epidermis $D_{0,thr}$ in response to CSC with R-134a and R-404a at various spurt durations: (a) $\tau_{CSC}=100$ ms, (b) $\tau_{CSC}=200$ ms, (c) $\tau_{CSC}=300$ ms.

$$We = \rho V_N^2 d / \sigma, \quad (2)$$

where ρ is droplet liquid density (kg/m^3), V_N is the droplet incident normal velocity (m/s), d is the incident droplet diameter (m), and σ is surface tension of incident droplet (mN/m). An *in vivo* study³³ with normal human skin revealed that when the Weber number of R-134a droplets was increased from 1100 to 5100, the threshold radiant exposure for nonspecific thermal injury to the epidermis did not increase, although the average scores of epidermal damage decreased, indicating the limitation of this means in terms of enhancing Q .

The second method to enhance Q is to increase the temperature difference between cryogen film and skin surface ($T_{skin,s} - T_{film}$), which is the driving force for heat transfer. The utilization of R-404a instead of R-134a is for this purpose. From the *in vitro* results, during cryogen spraying time, the temperature of R404-a film was approximately 13°C lower than that of R-134a film (-68°C versus -55°C). Increased ($T_{skin,s} - T_{film}$) subsequently resulted in increased heat removal from skin surface and also the temperature reduction within the superficial layers of skin, thus allowing higher threshold radiant exposures for irreversible thermal injury to the epidermis. This was evidenced by the *ex vivo* results of the

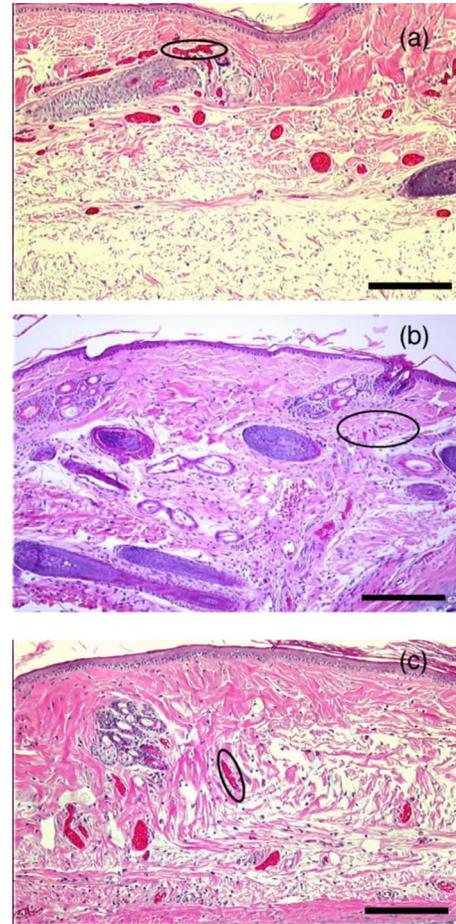


Fig. 8 Influence of CSC on the thermal injury to dermal blood vessels in rabbit ears: (a) no CSC, (b) 100 ms R-134a spurt, (c) 100 ms R-404a spurt. Ovals: the most superficial blood vessels that were thermally damaged. $D_0=13.5$ J/cm², $\lambda=585$ nm. Bars: 200 μm . The sites were excised 2 h after the laser irradiation.

normal dark human skin. In a previous study,³² we obtained an empirical relationship between Q and the difference in droplet temperature and the surface temperature (ΔT), droplet velocity (V_N), and diameter (d) for a poor-conducting surface sprayed with R-134a as

$$Q \propto \Delta T d^{0.13} V_N^{0.16}. \quad (3)$$

According to this relationship, Q is linearly related to ΔT and modestly dependent on d and V_N .

The third means to increase Q is to increase cryogen spurt duration τ_{CSC} . In the present *ex vivo* study, no obvious CSC-induced injury to the skin was observed when τ_{CSC} was up to 300 ms. However, in a previous study with a cultured *in vitro* model of human skin, CSC-induced injury was seen when τ_{CSC} was above 80 ms.³⁸ It may be possible that the differences in physical properties (e.g., water content) between the cultured *in vitro* model and human skin may contribute to the histological observations. Additionally, it may be possible that some types of injury that are detectable in the cultured *in vitro* model may not be observable in *ex vivo* human skin. In a clinical study, CSC-induced injury to the skin (hypopigmentation) was reported in a patient during hair removal.³⁹ Further

Table 2 Effect of CSC on the depth of thermally damaged blood vessels.

Site	Radiant Exposure (J/cm ²)	Cryogen Spurt Duration (ms)	Cryogen Type	Depth of Most Superficial Vascular Damage (μm)
1	10	100	R-134a	225
2	10	200	R-134a	170
3	10	300	R-134a	270
4	13.5	100	R-134a	165
5	13.5	200	R-134a	115
6	13.5	300	R-134a	235
7	10	100	R-404a	210
8	10	200	R-404a	110
9	10	300	R-404a	195
10	13.5	100	R-404a	270
11	13.5	200	R-404a	225
12	13.5	300	R-404a	180
13	10	0		125
14	13.5	0		70

studies are required to verify the safety of using long cryogen spurt duration. Furthermore, the *in vivo* study with rabbit’s external ears demonstrated that CSC with spurt durations above 100 ms increased the depth of the most superficial thermally damaged dermal blood vessels, implying that nonspecific cooling of rabbit ear vasculature might be caused by cryogen spurts with the settings applied. The appropriate τ_{CSC} for achieving spatial cooling selectivity depends on the depth of the targeted blood vessel.⁴⁰ For shallow targets (150 μm in depth), the optimum τ_{CSC} is predicted to be 170 to 300 ms; while for deep targets (400 μm in depth), the optimum τ_{CSC} is 400 ms.⁴⁰

In the present study, the injector-to-surface distance (85 mm) was selected based on the results of R-134a. We understand that although this distance is the optimized distance for R-134a, it may not be the one for R-404a. However, we have found in this study that use of R-404a results in additional heat removal even under “unoptimized” spraying conditions. Therefore, it would be reasonable to expect that heat removal could be further enhanced once the spraying parameters for R-404a are optimized.

Using a mathematical model,⁴¹ we investigated the thermal response of dark human skin (30% volumetric melanin content in the epidermis) with an embedded 500-μm thick blood layer to CSC (R-134a and R-404a) at $\tau_{CSC}=300$ ms in conjunction with 585-nm laser irradiation at the laser pulse duration of 1.5 ms and the radiant exposure of 4.2 J/cm². Figure 9 presents the predicted temperature and laser-induced thermal injury profiles (Arrhenius damage integral)⁴² immediately

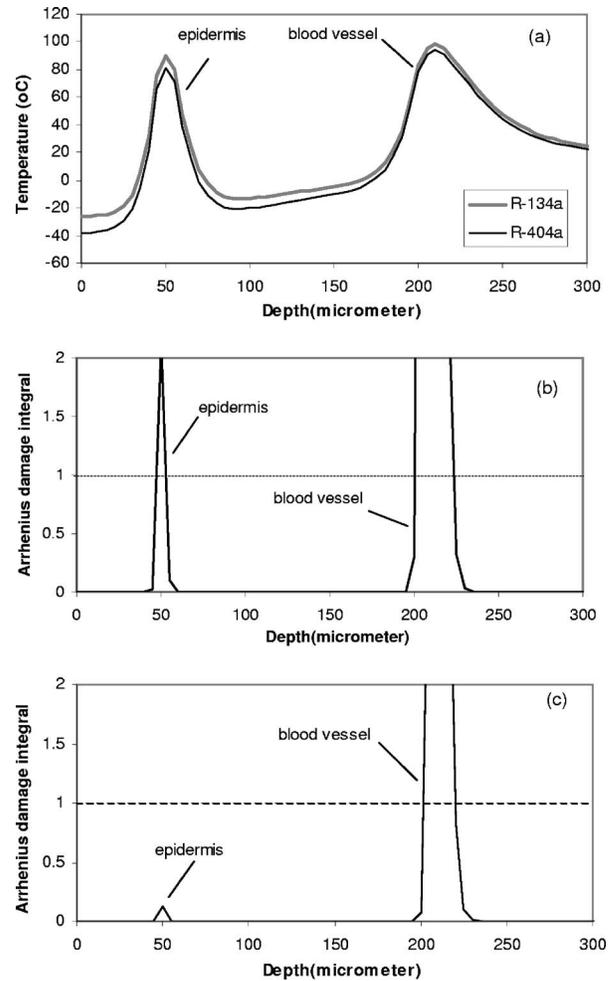


Fig. 9 Predicted spatial temperature and Arrhenius damage integral profiles of dark human skin (30% volumetric melanin content in the epidermis) with an embedded 500-μm thick blood layer in response to CSC (R-134a or R-404a) at $\tau_{CSC}=300$ ms in conjunction with 595-nm laser irradiation at $D_0=4.2$ J/cm² and $\tau_{laser}=1.5$ ms immediately after laser pulse termination: (a) temperature profiles, (b) laser-induced thermal injury profile when using a 300-ms R-134a spurt, (c) laser-induced thermal injury profile when using a 300-ms R-404a spurt. In (b) and (c), the dashed lines indicate the damage integral value of 1. When the damage integral reaches 1, then the tissue is assumed to be irreversibly damaged.

after the laser irradiation. When the damage integral reaches 1, then the tissue is assumed to be irreversibly damaged. With $\tau_{CSC}=300$ ms, the temperature rise of the epidermis immediately after the laser irradiation was 59°C for R-134a and 50°C for R-404a [Fig. 9(a), assuming skin initial temperature 30°C]. The comparative decreases of the temperature rise of the epidermis when using R-404a was approximately 15% in response to $\tau_{csc}=300$ ms. Nonspecific laser-induced thermal injury to the epidermis was predicted to occur when using R-134a [Fig. 9(b)], but the epidermis was predicted to be successfully protected when using R-404a [Fig. 9(c)].

In summary, CSC with R-404a increased the CSC-induced temperature reduction and the amount of heat removal from a skin phantom (demonstrated by the *in vitro* results). Therefore, it decreased the laser-induced peak temperature in the

epidermis (shown by the theoretical modeling results) and increased the threshold radiant exposures for irreversible thermal injury to the epidermis in dark human skin (evidenced by the *ex vivo* results). In view of these results, CSC using R-404a is expected to improve epidermal protection in dark human skin during cutaneous laser treatment.

5 Conclusions

CSC with R-404a resulted in greater amount of heat removal from a skin phantom in comparison to that with R-134a and increased the threshold radiant exposure for irreversible thermal injury to the epidermis in *ex vivo* dark human skin. *Ex vivo* results revealed that the utilization of R-404a spurts with the durations up to 300 ms did not induce obvious morphological changes to human skin. CSC with the spurt durations of 100 to 300 ms increased the depth of the most superficial thermally damaged dermal blood vessel compared with non-cooling, implying possible nonspecific cooling of superficial dermal blood vessels for both R-134a and R-404a.

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References

- R. Ashinoff and R. G. Geronemus, "Treatment of a port wine stain in a black patient with pulsed dye laser," *J. Dermatol. Surg. Oncol.* **18**, 147–148 (1992).
- M. Haedersdal, M. Gniadecka, J. Efsen, N. Bech-Thomsen, J. Keiding, and H. C. Wulf, "Side effects from the pulsed dye laser: the importance of skin pigmentation and skin redness," *Acta Derm Venereol* **78**, 445–450 (1998).
- K. Nouri, R. Romagosa, T. Chartier, L. Bowes, and J. M. Spencer, "Comparison of the 585 nm pulsed dye laser and the short pulsed CO₂ laser in the treatment of striae distensae in skin types IV and VI," *Dermatol. Surg.* **25**, 368–370 (1999).
- A. B. Garrett and S. Shieh, "Treatment of vascular lesions in pigmented skin with the pulsed dye laser," *J. Cutan Med. Surg.* **4**, 36–39 (2000).
- S. Sommer and R. A. Sheehan-Dare, "Pulsed dye laser treatment of port-wine stains in pigmented skin," *J. Am. Acad. Dermatol.* **42**, 667–671 (2000).
- T. S. Alster and E. L. Tanzi, "Laser surgery in dark skin," *Skinmed* **2**, 80–85 (2003).
- 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).
- 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).
- B. A. Buscher, T. O. McMeekin, and D. Goodwin, "Treatment of telangiectasia by using a long-pulse dye laser at 595 nm with and without dynamic cooling device," *Lasers Surg. Med.* **27**, 171–175 (2000).
- C. J. Chang and J. S. Nelson, "Cryogen spray cooling and higher fluence pulsed dye laser treatment improve port-wine stain clearance while minimizing epidermal damage," *Dermatol. Surg.* **25**, 767–772 (1999).
- C. H. Chiu, H. H. Chan, W. S. Ho, C. K. Yeung, and J. S. Nelson, "Prospective study of pulsed dye laser in conjunction with cryogen spray cooling for treatment of port wine stains in Chinese patients," *Dermatol. Surg.* **29**, 909–915 (2003).
- W. K. Woo, Z. F. Jasim, and J. M. Handley, "Evaluating the efficacy of treatment of resistant port-wine stains with variable-pulse 595-nm pulsed dye and 532-nm Nd:YAG lasers," *Dermatol. Surg.* **30**, 158–162 (2004).
- T. Kono, T. Yamaki, A. R. Ercocen, O. Fujiwara, and M. Nozaki, "Treatment of leg veins with the long pulse dye laser using variable pulse duration and energy fluences," *Lasers Surg. Med.* **35**, 62–67 (2004).
- T. Dai, B. M. Pikkula, J. W. Tunnell, D. W. Chang, and B. Anvari, "Thermal response of human skin epidermis to 595-nm laser irradiation at high incident dosages and long pulse durations in conjunction with cryogen spray cooling: an *ex-vivo* study," *Lasers Surg. Med.* **33**, 16–24 (2003).
- J. W. Tunnell, D. W. Chang, C. Johnston, J. H. Torres, C. W. Patrick Jr., M. J. Miller, S. L. Thomsen, and B. Anvari, "Effects of cryogen spray cooling and high radiant exposures on selective vascular injury during laser irradiation of human skin," *Arch Dermatol.* **139**, 743–750 (2003).
- T. B. Fitzpatrick, "The validity and practicality of sun-reactive skin types I through VI," *Arch Dermatol.* **124**, 869–871 (1988).
- B. Anvari, B. S. Tanenbaum, T. E. Milner, K. Tang, L. H. Liaw, K. Kalafus, S. Kimel, and J. S. Nelson, "Spatially selective photocoagulation of biological tissues: a feasibility study utilizing cryogen spray cooling," *Appl. Opt.* **35**, 3314–3320 (1996).
- B. Anvari, B. S. Tanenbaum, W. Hoffman, S. Said, T. E. Milner, L. H. Liaw, and J. S. Nelson, "Nd:YAG laser irradiation in conjunction with cryogen spray cooling induces deep and spatially selective photocoagulation in animal models," *Phys. Med. Biol.* **42**, 265–282 (1997).
- B. Anvari, B. J. Ver Steeg, T. E. Milner, B. S. Tanenbaum, T. J. Klein, E. Gerstner, S. Kimel, and J. S. Nelson, "Cryogen spray cooling of human skin: effects of ambient humidity level, spraying distance, and cryogen boiling point," *Proc. SPIE* **3192**, 106–110 (1997).
- C. J. Chang, B. Anvari, and J. S. Nelson, "Cryogen spray cooling for spatially selective photocoagulation of hemangiomas: a new methodology with preliminary clinical reports," *Plast. Reconstr. Surg.* **102**, 459–463 (1998).
- S. Akdemir and S. Arin, "Determination of cooling effect of R12 and R404A," *J. Biol. Sci.* **3**, 1114–1125 (2003).
- B. M. Pikkula, J. H. Torres, J. W. Tunnell, and B. Anvari, "Cryogen spray cooling: Effects of droplet size and spray density on heat removal," *Lasers Surg. Med.* **28**, 103–112 (2001).
- F. A. Duck, *Physical Properties of Tissue*, Academic Press, London (1990).
- J. H. Torres, J. S. Nelson, B. S. Tanenbaum, T. E. Milner, D. M. Goodman, and B. Anvari, "Estimation of internal skin temperatures in response to cryogen spray cooling: Implications for laser therapy of port wine stains," *IEEE J. Sel. Top. Quantum Electron.* **5**, 1058–1066 (1999).
- J. H. Torres, J. W. Tunnell, B. M. Pikkula, and B. Anvari, "An analysis of heat removal during cryogen spray cooling and effects of simultaneous airflow application," *Lasers Surg. Med.* **28**, 477–486 (2001).
- J. W. Tunnell, J. H. Torres, and B. Anvari, "Methodology for estimation of time-dependent surface heat-flux due to cryogen spray cooling," *Ann. Biomed. Eng.* **30**, 19–33 (2002).
- E. Karapetian, G. Aguilar, S. Kimel, E. J. Lavernia, and J. S. Nelson, "Effects of mass flow rate and droplet velocity on surface heat flux during cryogen spray cooling," *Phys. Med. Biol.* **48**, N1–N6 (2003).
- G. Aguilar, G. X. Wang, and J. S. Nelson, "Dynamic behavior of cryogen spray cooling: effects of spurt duration and spray distance," *Lasers Surg. Med.* **32**, 152–159 (2003).
- G. Aguilar, G. X. Wang, and J. S. Nelson, "Effect of spurt duration on the heat transfer dynamics during cryogen spray cooling," *Phys. Med. Biol.* **48**, 2169–2181 (2003).
- W. Jia, G. Aguilar, G. X. Wang, and J. S. Nelson, "Heat-transfer dynamics during cryogen spray cooling of substrate at different initial temperatures," *Phys. Med. Biol.* **49**, 5295–5308 (2004).
- G. Aguilar, H. Vu, and J. S. Nelson, "Influence of angle between the nozzle and skin surface on the heat flux and overall heat extraction during cryogen spray cooling," *Phys. Med. Biol.* **49**, N147–N153 (2004).
- B. M. Pikkula, J. W. Tunnell, D. W. Chang, and B. Anvari, "Effects of droplet velocity, diameter, and film height on heat removal during cryogen spray cooling," *Ann. Biomed. Eng.* **32**, 1131–1140 (2004).

33. B. M. Pikkula, D. W. Chang, T. Dai, and B. Anvari, "Epidermal and vascular damage analysis of in vivo human skin in response to 595 nm pulsed laser irradiation," *Lasers Surg. Med.* **37**, 19–28 (2005).
34. M. J. C. Van Gemert, A. J. Welch, J. W. Pickering, and O. T. Tan, "Laser treatment of port wine stains," in *Optical-Thermal Response of Laser-Irradiated Tissue*, A. J. Welch, M. J. C. van Gemert, Eds, pp. 789–830, New York, Plenum Press (1995).
35. R. F. Woolson, *Statistical Methods for the Analysis of Biomedical Data*, Wiley & Sons, Inc., New York (1987).
36. J. E. Eckhause and R. D. Reitz, "Modeling heat transfer to impinging fuel sprays in direct-injection engines," *Atomization Sprays* **5**, 213–242 (1995).
37. J. Schmidt and H. Boye, "Influence of velocity and size of the droplets on the heat transfer in spray cooling," *Chem. Eng. Technol.* **24**, 255–260 (2001).
38. B. Kao, K. M. Kelly, G. Aguilar, Y. Hosaka, R. J. Barr, and J. S. Nelson, "Evaluation of cryogen spray cooling exposure on in vitro model human skin," *Lasers Surg. Med.* **34**, 146–154 (2004).
39. R. J. Hirsch, W. A. Farinelli, and R. R. Anderson, "A closer look at dynamic cooling," *Lasers Surg. Med.* **30**(S14), 36 (2002).
40. W. Verkruysse, B. Majaron, B. S. Tanenbaum, and J. S. Nelson, "Optimal cryogen spray cooling parameters for pulsed laser treatment of port wine stains," *Lasers Surg. Med.* **27**, 165–170 (2000).
41. T. Dai, B. M. Pikkula, L. V. Wang, and B. Anvari, "Comparison of human skin opto-thermal response to near-infrared and visible laser irradiations: a theoretical investigation," *Phys. Med. Biol.* **49**, 4861–4877 (2004).
42. J. Pearce and S. L. Thomsen, "Rate process analysis of thermal damage," in *Optical-Thermal Response of Laser Irradiated Tissue*, A. J. Welch and M. J. van Gemert, Eds, pp. 561–606, New York, Plenum Press (1995).