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Thermal Responses of Ex Vivo Human Skin During Multiple Cryogen Spurts and 1,450 nm Laser Pulses

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Background and Objective: Although cryogen spray cooling (CSC) is used to minimize the risk of epidermal damage during laser dermatologic surgery, concern has been expressed that CSC may induce cryo-injury. The objective of this study is to measure temperature variations at the epidermal–dermal junction in ex vivo human skin during three clinically relevant multiple cryogen spurt—laser pulse sequences (MCS-LPS).

Study Design/Materials and Methods: The epidermis of ex vivo human skin was separated from the dermis and a thin-foil thermocouple (13 μm thickness) was inserted between the two layers. Thermocouple depth and epidermal thickness were measured using optical coherence tomography (OCT). Skin specimens were preheated to 30°C before the MCS-LPS were initiated. Three MCS-LPS patterns, with total cryogen spray times of 38, 30, and 25 milliseconds respectively, were applied to the specimens in combination with laser fluences of 10 and 14 J/cm^2, while the thermocouple recorded the temperature changes at the epidermal–dermal junction.

Results: The thermocouple effectively recorded fast temperature changes during three MCS-LPS patterns. The lowest temperatures measured corresponded to the sequences with longer pre-cooling cryogen spurts. No sub-zero temperatures were measured for any of the MCS-LPS patterns under study.

Conclusions: The three clinically relevant MCS-LPS patterns evaluated in this study do not cause sub-zero temperatures in ex vivo human skin at the epidermal–dermal junction and, therefore, are unlikely to cause significant cryogen induced epidermal injury. Lasers Surg. Med. 38:137–141, 2006. © 2006 Wiley-Liss, Inc.

Key words: epidermal–dermal junction temperature; cryogen injury

INTRODUCTION

Cryogen spray cooling (CSC) is an efficient method of selective epidermal cooling and has been used in conjunction with laser therapy for various dermatoses, such as vascular lesions [1–3], hair removal, and non-ablative skin rejuvenation [4]. By preventing excessive epidermal heating, CSC decreases treatment pain, allows the safer use of higher laser radiant exposures, permits treatment of patients with darker skin types, and enhances therapeutic outcome. More recently, multiple cryogen spurt and laser pulse sequences (MCS-LPS), which consist of several cryogen spurts and laser pulses in an intermittent pattern, have been incorporated into some therapeutic laser devices.

A diode laser with a wavelength of 1,450 nm in combination with CSC is clinically used for treatment of acne, acne scarring, and non-ablative photorejuvenation [5–7]. The laser light is used to target the dermal sebaceous glands and associated structures, while CSC protects the epidermis.

Previous in vitro [8,9] and in vivo [10] studies have suggested that multiple cryogen spurts (MCS) are safe. Despite the data presented in these studies, concerns have been expressed that use of MCS is more likely to induce cryo-injury than the traditional approach of a single cryogen spurt. However, these studies [8–10] only involved the exposure of the skin to cryogen without laser irradiation, which is inconsistent with current clinical use where the skin is exposed to both CSC and laser irradiation.

Fast-response temperature sensors, such as thermocouples, have been used to measure temperature changes and estimate the total heat removed from the surface during CSC [11–15]. When the thermocouple time constant is much shorter than the thermal relaxation time of the surrounding tissue, artifacts induced by direct absorption of laser irradiation by the thermocouple can be removed by applying exponential and linear fits to measured temperature curves [16]. This technique allows fast-response thermocouples to measure temperature variations induced by MCS-LPS patterns.

The objective of the work described herein is to measure temperature dynamics at the epidermal–dermal junction in ex vivo human skin specimens using fast-response thermocouples during three clinically relevant MCS-LPS. Based on the previous MCS-LPS literature [6,10], the

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working hypothesis is that the epidermal–dermal junction will not reach sub-zero temperatures in response to clinically relevant MCS-LPS patterns.

MATERIALS AND METHODS

Skin Specimen Preparation

Two pieces of cryopreserved human skin of low melanin concentration–type I or II (Community Tissue Services, Dayton, OH) from different sources were thawed and cut into 2 mm thick specimens approximately 1×1 cm^2 in area. Specimens were soaked in a 1:1 mixture solution of dispase and saline without Ca^{2+} for 90–120 minutes at room temperature until the epidermis and dermis were physically separated. Specimens were carefully removed from the solution and subsequently placed on a 1 cm thick agar gel phantom (1.5 g agar powder per 100 ml water) formed in Petri dishes, which acted as a carrier base. Petri dishes containing the skin specimens on top of the phantom were slowly heated with a hot plate to 30°C, to mimic in vivo human skin temperature. This slow heating process also removed excess water in the specimen. Although water loss could be substantial during the heating process, all samples were exposed to MCS-LPS 30 seconds after the specimen reached 30°C, insuring identical initial conditions for all samples.

Temperature Sensor

A 13 μm thick type-K thin-foil thermocouple (OMEGA® Cement-On Style 2, Omega Engineering, Inc., Stamford, CT) was inserted between the separated epidermis and dermis, as shown in Figure 1. The thermocouple response time is 2–5 milliseconds, its absolute temperature accuracy is ± 1°C and a good thermal contact on both sides of the thermocouple is expected due to the high moisture content at the epidermal–dermal junction. With all these conditions combined we estimate an uncertainty in the temperature measurements of 10%–12%. An optical coherence tomography (OCT) system [17,18] was utilized to image the skin cross section to measure thermocouple depth and epidermal thickness.

Laser Device

A 1,450-nm mid-infrared diode laser (Smoothbeam™, Candela Corporation, Wayland, MA) equipped with a CSC device was used for this study. The laser spot diameter was 6 mm. Three clinically relevant MCS-LPS patterns [5–7,19–22] were applied on the skin specimens. As illustrated in Figure 2, MCS-LPS-1 involved the longest total cryogen spray time ($t_{CSC}{}^1 = 38$ milliseconds). MCS-LPS-2 ($t_{CSC}{}^2 =$ 30 milliseconds) involved both shorter pre-cooling (5 milliseconds) and intermittent (5 milliseconds) cryogen spurts as compared to MCS-LPS-1. MCS-LPS-3 ($t_{CSC}{}^3 =$ 25 milliseconds) was similar to MCS-LPS-2, except that there was no pre-cooling spurt. Laser fluences of 10 and 14 J/cm^2 were employed with each MCS-LPS pattern.

RESULTS

Figure 3 shows representative OCT images of the cross-sections of the two skin specimens. The two short white lines in the image are due to light reflection from the thermocouple inserted between the epidermis and dermis. Thus, by measuring the distance from the skin surface to the thermocouple, the epidermal thickness could be determined. The skin specimen in Figure 3a had a thinner epidermis (~50 μm), than that shown in Figure 3b (~100 μm). Resolution of the OCT system was ~10 μm.

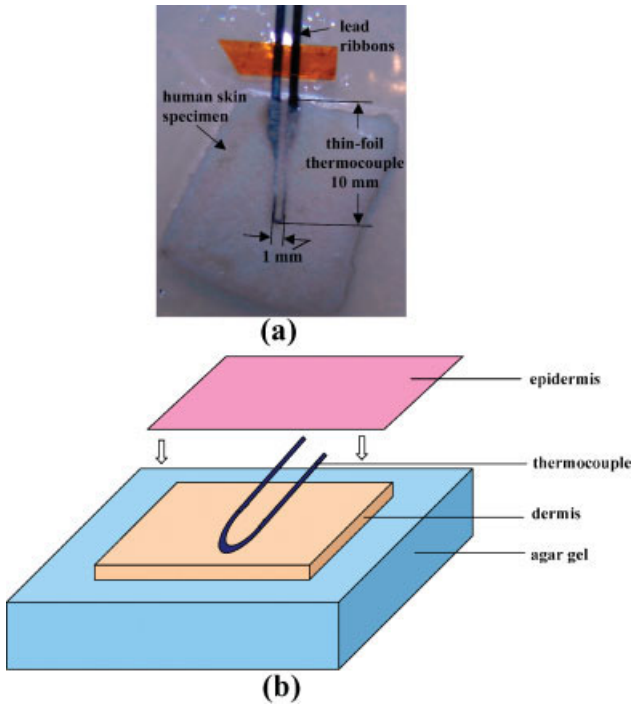

Fig. 1. Photo (**a**) and schematics (**b**) show how ex vivo human skin specimens were placed on top of an agar gel phantom. A thick type-K thin-foil thermocouple was inserted between the separated epidermis and dermis.

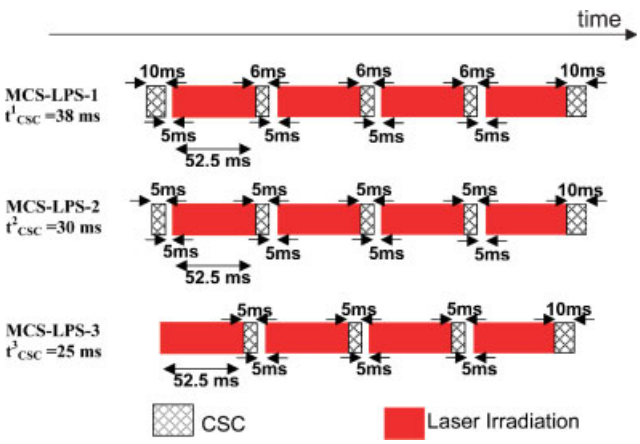

Fig. 2. Three clinically relevant MCS-LPS patterns. MCS-LPS-1 involved the longest total cryogen spray time ($t_{CSC}{}^1 =$ 38 milliseconds). MCS-LPS-2 ($t_{CSC}{}^2 = 30$ milliseconds) involved both shorter pre-cooling (5 milliseconds) and intermittent (5 milliseconds) cryogen spurts as compared to MCS-LPS-1. MCS-LPS-3 ($t_{CSC}{}^3 = 25$ milliseconds) was similar to MCS-LPS-2, except that there was no pre-cooling spurt.

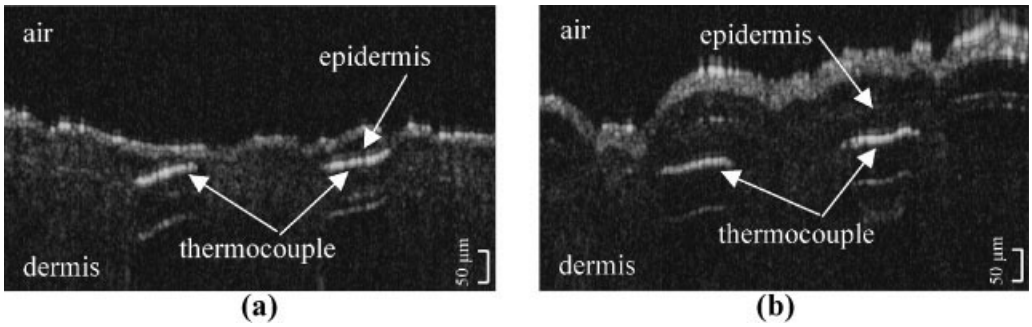

Fig. 3. OCT images of the cross-sections of the two skin specimens. The two short white lines in the image are due to light reflection from the thermocouple. The specimen in (a) had a thinner epidermis (~50 μm), than that shown in (b) (~100 μm).

Figures 4–6 show representative temperature measurements obtained with the thin-foil thermocouples for the three different MCS-LPS patterns at two different epidermal thickness (~50 μm as in Figs. 4a–6a; ~100 μm as in Figs. 4b–6b) in response to two different laser fluences (10 and 14 J/cm^2). Three experiments were conducted for each set of MCS-LPS patterns. The variation in the thermal response was within 5%.

Figures 4 and 5 indicate that for MCS-LPS-1 and -2, both of which include a pre-cooling spurt, temperatures at both depths of 50 (Figs. 4a and 5a) and 100 μm (Figs. 4b and 5b) first decreased at the onset of the MCS-LPS patterns. MCS-LPS-1 induced even larger temperature decreases than MCS-LPS-2 due to the longer pre-cooling and intermittent cryogen spurts. For both MCS-LPS-1 and -2, larger temperature decreases were measured at depths of 50 μm as compared to 100 μm.

With MCS-LPS-3 (Fig. 6), which did not include a pre-cooling spurt, temperatures increased immediately after irradiation by the first laser pulse. Figure 6 also show that the highest maximum temperatures were measured with MCS-LPS-3 as compared to MCS-LPS-1 and -2 (Figs. 4 and 5). No sub-zero temperatures were measured for any of the MCS-LPS patterns under study.

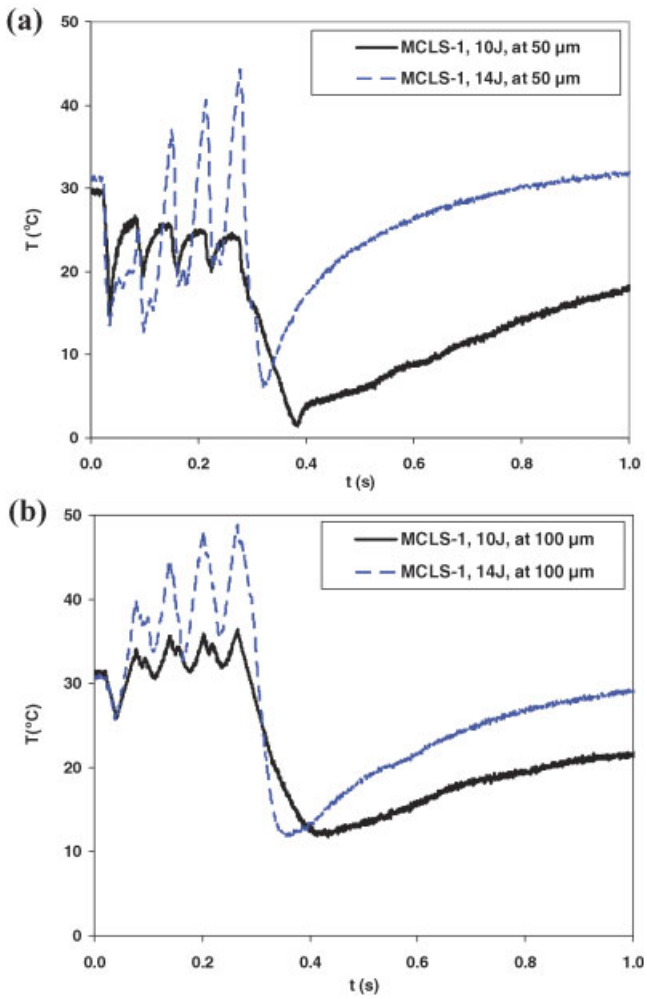

Fig. 4. Representative temperature measurements of MCS-LPS-1 obtained by the thermocouples at two different epidermal thicknesses ((**a**) 50 μm and, (**b**) 100 μm) in response to two different laser fluences (10 and 14 J/cm^2). [Figure can be viewed in color online via www.interscience.wiley.com.]

DISCUSSION

In this study, we measured temperature dynamics at the epidermal–dermal junction in ex vivo human skin specimens using fast-response thermocouples during three clinically relevant MCS-LPS. Our data support the working hypothesis that the epidermal–dermal junction would not reach sub-zero temperatures in response to clinically relevant MCS-LPS patterns.

Thermocouple Artifact

The artifact created by possible direct thermocouple absorption of laser irradiation can be ignored in the current study for the following reasons: (1) by applying the same technique as in [16], the maximum error induced by thermocouple artifact is only 2–4°C, which is small compared to the total temperature increase during each laser pulse; and (2) as the objective of the current study is evaluation of potential cryo-injury at the epidermal–dermal junction, accurate measure-

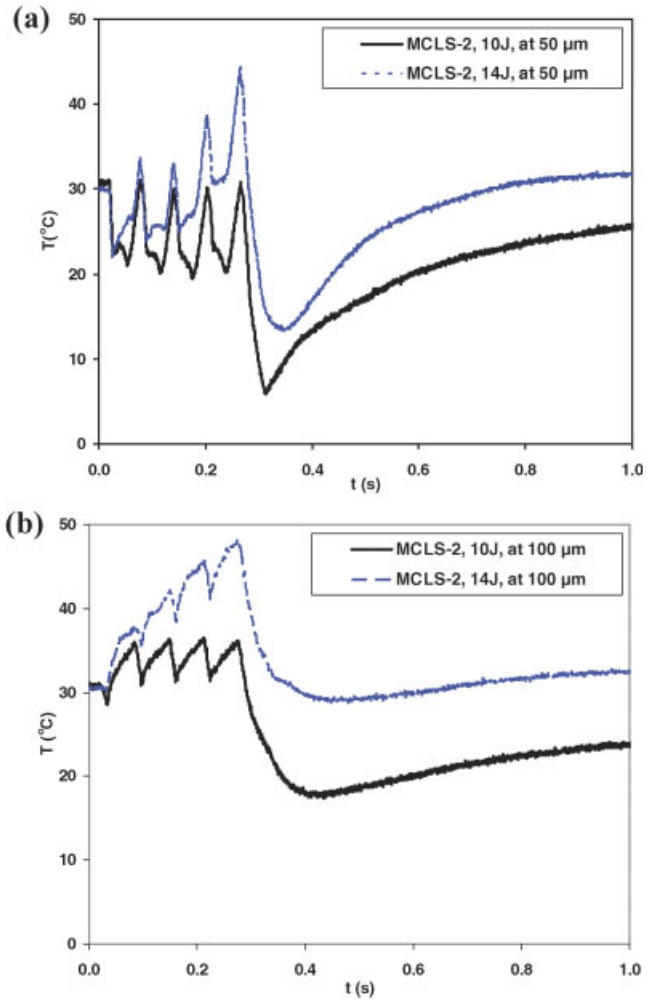

Fig. 5. Representative temperature measurements of MCS-LPS-2 obtained by the thermocouples at two different epidermal thicknesses (**a**) 50 μm and, (**b**) 100 μm in response to two different laser fluences (10 and 14 J/cm^2). [Figure can be viewed in color online via www.interscience.wiley.com.]

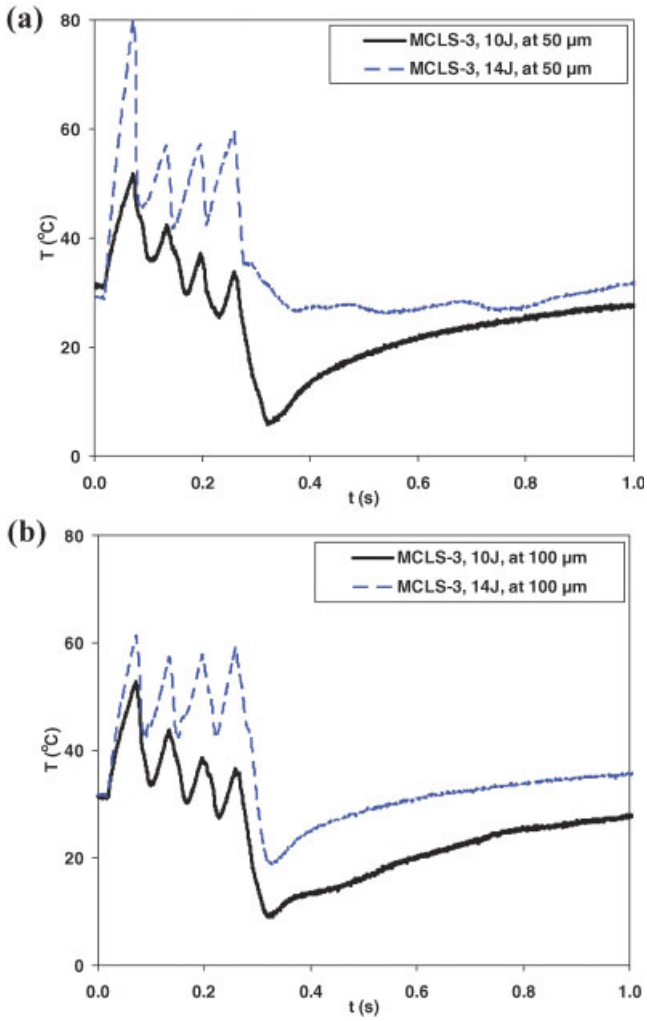

Fig. 6. Representative temperature measurements of MCS-LPS-3 obtained by the thermocouples at two different epidermal thicknesses ((**a**) 50 μm, and (**b**) 100 μm) in response to two different laser fluences (10 and 14 J/cm^2). [Figure can be viewed in color online via www.interscience.wiley.com.]

ments of maximum temperatures during laser irradiation are less crucial. Furthermore, the thermocouple response time is 2–5 milliseconds, while the cooling time, including the cryogen spurt time and delay time, is at least 10 milliseconds for each cryogen spurt of all three MCS-LPS patterns. Thus, the cooling time is 2–5 times the thermocouple response time, indicating that the minimum epidermal–dermal junction temperature was accurately measured.

Ex vivo Human Skin Specimen

The use of ex vivo human skin specimens serves as a model that closely approximates in vivo human skin as compared to other previously used models such as an epoxy phantom [13], Plexiglas® [12,14], and skin-equivalent engineered tissue [23,24]. Furthermore, use of ex vivo human skin allowed insertion of the thermocouple at the epidermal–dermal junction, which is very difficult to accomplish in vivo. However, differences between ex vivo and in vivo human skin should be noted. First, there was no blood flow in the ex vivo skin specimen, which would provide a heat source for the epidermis and, presumably, mitigate any potential cryo-injury [23]. Second, although we carefully warmed the skin specimen and visually insured its dryness to mimic in vivo skin, we expect that the water content in ex vivo skin would differ from that found in vivo, which may result in different thermal properties and, thus, cooling dynamics.

Insulating Effect of the Skin

A previous study [11] using a Plexiglas® model involved measurements of surface temperature during cryogen spurts of different durations and nozzle-to-skin distances. The results demonstrated that by applying a single spurt of 15 milliseconds at a spray distance of 25 mm, the surface temperature dropped from 20°C (room temperature) to −26.2°C (boiling temperature of the cryogen) and remained

at that level for 60 milliseconds in the absence of an external heat source. Although the temperature at the surface decreases rapidly due to direct contact with the impinging cryogen droplets, temperature changes are mitigated at the epidermal–dermal junction due to the insulating effect of the epidermis.

Future Work

Since no sub-zero temperatures were measured during any of the MCS-LPS patterns under study, the three clinically relevant MCS-LPS patterns are unlikely to cause significant cryogen-induced epidermal injury. However, because there is inadequate information on the requisite degree of exposure to low temperatures that causes temporary or permanent injury to keratinocytes and melanocytes, it is possible that, under certain conditions, mild temperature reductions could induce injury for patients with darker skin types or more sensitive skin. Further investigation is warranted to address this important issue.

CONCLUSIONS

Temperature variations were measured at the epidermal–dermal junction of ex vivo human skin specimens using thin-foil thermocouples during application of three clinically relevant MCS-LPS patterns, in combination with laser fluences of either 10 or 14 J/cm^2. No sub-zero temperatures were measured during any of the MCS-LPS patterns under study. Thus, the three clinically relevant MCS-LPS patterns are unlikely to cause significant cryogen induced epidermal injury.

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