Effects of Skin Temperature on Lesion Size in Fractional Photothermolysis

Hans Laubach, MD,1 Henry H. Chan, MD,2 Francisca Rius, PhD,3,4 R. Rox Anderson, MD,1 and Dieter Manstein, MD1*

1Wellman Laboratories for Photomedicine and Department of Dermatology, Massachusetts General Hospital and Harvard Medical School, Boston, MA. E-mail: Dmanstein@partners.org
2University of Hong Kong and Chinese University of Hong Kong, Hong Kong
3University of Malaga, Malaga, Spain

Background and Objectives: Fractional photothermolysis is a new concept in cutaneous re-modeling whereby laser-induced microscopic zones of thermal injury (MTZ—Microscopic Treatment Zones) are surrounded by normal, viable tissue. This unique thermal damage pattern allows re-epithelialization in less than 24 hours. To increase patient comfort level during the procedure of fractional photothermolysis, simultaneous skin cooling has been proposed and is now extensively used. The purpose of this in vitro study was to examine the influence of skin temperature on the diameter of the epidermal microthermal zone and the extent of thermal injury per unit area.

Materials and Methods: Fractional photothermolysis was performed with a 1,550 nm fiber laser (Fraxel SR Laser) with 10 mJ per pulse on full-thickness cadaver skin. The skin samples were brought prior to exposure to temperatures that ranged from 0 to 45°C. The epidermis of the skin samples was separated by dispase treatment, stained for thermal damage by NBTC stain, and lesion diameter was assessed by a blinded investigator.

Results: The average MTZ diameter exhibits a positive, linear relationship with skin temperature (R² = 0.904, P < 0.0001). As the skin temperature increases from 0 to 45°C, the MTZ diameter increases from 93 to 147 µm (58%), and the MTZ area from 6,870 to 17,050 µm² (148%).

Conclusion: The skin temperature affects the size of epidermal MTZs during fractional photothermolysis and is an important variable factor. The use of simultaneous skin cooling increases patient comfort; however, as it also decreases MTZ size, it may interfere with treatment efficacy. The control of skin temperature is necessary to provide a consistent outcome and to be able to compare treatments.


© 2007 Wiley-Liss, Inc.

Key words: temperature; lesion size; MTZ; fractional photothermolysis; fractional

BACKGROUND

Fractional photothermolysis is a new concept in cutaneous re-modeling [1], in which multiple zones of microthermal injury, called Microscopic Treatment Zones (MTZs), are generated in the skin by focused laser irradiation. MTZs consist of sharply confined tissue denaturation that is surrounded by viable tissue. The unique thermal damage pattern of laser-assisted fractional photothermolysis allows for re-epithelialization in less than 24 hours without loss of epidermal barrier functioning [1]. This approach reduces substantially the down time and the complications that are associated with traditional ablative laser skin resurfacing.

During each treatment session, a variable ratio of the skin surface is directly exposed to thermal injury. The epidermal injury of each MTZ is confined to a microscopically small area, and the migration from surrounding epidermal keratinocytes allows re-epithelialization in less than 24 hours. This rapid healing process of the epidermal MTZs significantly decreases the risk of complications. The size of the MTZs can be determined by the energy that is delivered for each MTZ. In typical clinical settings, approximately 10–20% of the skin surface is directly damaged.

Currently fractional photothermolysis is performed 1 hour after the application of a customized topical anesthetic cream. For certain patients and settings, it may be a clinical challenge to provide sufficient analgesia with topical lidocaine alone. Simultaneous skin cooling has been known to decrease pain perception during CO2 laser resurfacing [2], and hence simultaneous skin cooling during fractional photothermolysis with a device that delivers a constant flow of cold air (Cryo 5, Zimmer Medizin Systems, Irvine, CA) has been proposed. It is now extensively used to decrease the patient’s discomfort. Although simultaneous skin cooling in fractional photothermolysis is not used for epidermal sparing, as it is in non-ablative skin remodeling, it decreases the risk of bulk tissue heating is commonly seen with multiple passes, and therefore simultaneous cooling increases. This increases

*Correspondence to: Dieter Manstein, MD, Wellman Laboratories for Photomedicine, Department of Dermatology, Harvard Medical School, Boston, MA. E-mail: Dmanstein@partners.org

Accepted 2 October 2006

Published online 29 January 2007 in Wiley InterScience (www.interscience.wiley.com).

DOI 10.1002/lsm.20453

© 2007 Wiley-Liss, Inc.
the safety of the treatment. This has led to the increased use of simultaneous skin cooling though the full effects of a decrease in skin temperature during fractional photothermolysis remain uninvestigated at this time.

The purpose of this in vitro study is to look at the influence of skin temperature on the diameter of the epidermal MTZ diameter and the total extent of the thermally damaged surface area.

The results of this study will allow a better understanding of the interaction between skin surface cooling and the thermal damage pattern obtained during fractional photothermolysis.

MATERIALS AND METHODS

Previously frozen human cadaver skin was placed on wet gauze on a thermal plate (Cole-Parmer, Vernon Hills, IL) allowing to maintain the samples at various preset temperatures. Optiguide Blue (Reliant, Palo Alto, CA) and a thin layer of 30% lidocaine ointment were applied to the skin before laser exposure, and the skin surface temperature was monitored by an infrared thermometer. Laser exposure was performed at skin temperatures of 0, 5, 10, 15, 20, 25, 30, 35, 37, 40, and 45 C. Each exposure consisted of two passes with a Fraxel® laser (Reliant) at an energy of 10 mJ/MTZ and a density of 250 MTZ/cm² per pass. The skin temperature was measured before and immediately after each exposure to verify the precise skin temperature during the exposure.

After each laser exposure, 6-mm punch biopsies were performed at the center of the exposed area and were sent for histological assessment. The biopsies were immersed in dispase solution (Sigma-Aldrich Chemical Company, St. Louis, MO) as described by Okada et al. [3], and stored at 4°C overnight to allow the epidermis to detach from the dermis. Nitro-Blue-Tetrazolium-Chloride (NBTC) stain was applied to the epidermis and the tissue was again allowed to incubate overnight at 4°C [4]. The NBTC staining method allows the monitoring of the tissue necrosis that is caused by the irreversible thermal alteration of essential mitochondrial enzymes. It differentiates viable regular cells from thermally damaged cells by staining the first blue by an enzymatic reaction of unaltered mitochondrial protein and leaving the latter unstained to establish a well-demarcated zone of epidermal necrosis (Fig. 1).

The epidermis was then examined under a light microscope, and an investigator who was blinded to the details of the experiment assessed the diameter of 48 randomly selected MTZs for each temperature under investigation (48 lesions were chosen because this was the minimum amount of MTZs that was available for analysis in all of the samples). Two lesion diameters that were perpendicular to each other were measured for each epidermal MTZ. Overlapping MTZs, which could be seen with multiple passes, were excluded from the measurements by the investigator. For statistical analysis, we calculated the mean of the MTZ diameter and its standard deviation, and analyzed the surface area per MTZ that was thermally damaged. This allowed a more robust measurement of diameter and area since multiple samples were used to estimate the mean values.

Statistical Analysis

Linear regression analysis was applied to determine the relationship between average MTZ area and average damaged surface area and skin surface temperature. The coefficient of determination ($R^2$) was used to indicate the strength of the linear relationships using skin temperature as the predictor and also as a measure for describing how much variability in diameter and area was explained by skin temperature. Finally, the $R^2$ coefficient was used to quantify the magnitude of the linear relationship. Regression equations were derived using the method of least-squares to estimate the slope and y-intercept of the fitted linear models. The analysis was checked for normality, collinearity, and serial correlation, and no violations were found. Statistical analysis was performed using the SPSS software package (version 14.0, SPSS, Inc., Chicago, IL). All two-tailed values of $P < 0.05$ were considered statistically significant.

RESULTS

The relationship of the average diameter of the MTZs at different skin surface temperatures is shown in Figure 2. A linear regression graph is superimposed over the empirical data points. The MTZ diameter increases from 93 to 147 μm, or by 58%, as the temperature rises from 0°C (Fig. 3) to 45°C (Fig. 4). The linear model has an excellent fit to the data ($R^2 = 0.904, P < 0.0001$), which indicates that...
there is a positive linear correlation between the diameter of the MTZ and the skin surface temperature. The derived linear regression equation for the device (1550 nm Fraxel SR® Laser) for a pulse:

\[ D_{MTZ}(T_{surf}) = D_0 + C_D \cdot T_{surf} \]

\( T_{surf} \) = Temperature [°C] at skin surface. \( D_{MTZ} \) = Mean MTZ diameter [μm] at \( T_{surf} \). \( D_0 \) = Mean MTZ diameter at 0°C = 95 μm. \( C_D \) = Slope Coefficient (Fraxel SR® at 10 mJ) = 1.02 μm °C⁻¹.

The relationship of the average MTZ area at different skin surface temperatures is shown in Figure 5. A linear and a quadratic regression graph are superimposed over the empirical data points. There is an increase of MTZ area from 6,870 to 17,050 μm² (148%), as the temperature rises from 0 to 45°C. The magnitude of the linear relationship is strong and the fit to the actual data is good (\( R^2 = 0.885, P < 0.0001 \)). The quadratic model (\( R^2 = 0.917, P < 0.0001 \)) provides a marginally better fit. As for a clinically relevant
temperature range, the linear model allows for a good and simple fit, we have provided the general form of the linear regression equation with the derived parameters:

\[
A_{MTZ}(T_{surf}) = A_0 + C_A \times T_{surf}
\]

\[
T_{surf} = \text{Temperature } [{}^\circ \text{C}] \text{ at skin surface. } A_{MTZ} = \text{Mean MTZ area } [\mu m^2] \text{ at } T_{surf}, A_0 = \text{Mean MTZ area at } 0 \degree C = 6,838 \mu m^2. C_A = \text{Slope coefficient (Fraxel SR}^R\text{C at } 10 \text{ mJ) } = 191.8 \mu m^2/{^\circ} \text{C}.
\]

**DISCUSSION**

Skin rejuvenation with different types of lasers and light sources has generated much interest in the past few decades. Non-ablative dermal remodeling and ablative skin resurfacing are currently well-established treatment modalities for this indication. Conventional ablative skin resurfacing with CO\(_2\) or Er:YAG laser removes the entire epidermis of the treatment area, and is generally considered to be the most effective laser treatment option for the repair of photodamaged tissue. However, patients sometimes experience significant side effects after the treatment. Long-lasting erythema and pigmentedary changes, infection, and scarring are particularly unacceptable for many patients [5–8].

Non-ablative laser and intense pulse-light systems are currently used as an alternative for tightening the skin without harming the epidermis. In this technique, controlled dermal heating without epidermal damage is achieved by combining the laser or intense pulsed-light treatment with the simultaneous cooling of the skin surface [9]. The selective thermal damage of the dermis is followed by a wound healing response from the damaged tissue, which ultimately leads to new collagen formation. The absence of epidermal damage decreases the severity and degree of side effects that are associated with the treatment [10–15]. Although most studies have indicated significant levels of patient satisfaction and even histological and biochemical evidence of improvement, photographic assessment by blinded observers has often found only minimal or no improvement. Thus, limited clinical efficacy is the major disadvantage of this technique compared to ablative laser skin resurfacing [16,17].

Fractional photothermolysis was introduced by Manstein et al. [1] as a new concept in laser skin rejuvenation. To decrease the discomfort of patients during the procedure, simultaneous skin cooling has been proposed and is now extensively used, although the effect of different skin temperatures on fractional photothermolysis has not been further investigated.

In this study, we investigated the effect of tissue temperature on epidermal MTZ diameter and area during fractional photothermolysis. We found that skin temperature has a direct positive linear correlation with the MTZ diameter, and also with the MTZ area for the clinically relevant temperature range. When the skin temperature is decreased by body temperature by simultaneous skin cooling to 20°C, there is a corresponding decrease in the MTZ area of approximately 40%. Hypothetically, an appropriate increase of energy per MTZ could be used to overcome the decrease in MTZ diameter that is associated with a well-determined decrease in skin surface temperature. However, the extent to which the change in the thermally damaged surface area that is caused by changes in skin temperature alters the clinical outcome remains unclear, and must be further investigated. Until such clinical studies have been carried out, skin temperature should be acknowledged as an important variable in the treatment, in addition to energy per MTZ, number of passes, and MTZ density delivered. Clinical studies should particularly control and monitor skin temperature to allow for easy and meaningful comparisons. Hammes et al. investigated the effects of simultaneous skin cooling on the treatment of facial telangiectasias with PDL [18]. The skin temperatures investigated were 20 and 17°C, which were achieved by a constant flow of cold air, and one additional control site without any skin cooling was used as a reference. Hammes et al. showed that although cooling the skin to 17°C provided better analgesia, it also interfered significantly with the efficacy of the treatment, and thus 20°C is a better compromise between analgesia and treatment efficacy. The extent to which different degrees of skin cooling during fractional photothermolysis affect patient analgesia remains unclear, and the optimum combination of skin cooling and energy delivered to attain the maximum clinical efficacy with a minimum of patient discomfort remains to be determined.

Our findings also apply in general to other infrared laser devices, which produce a fractional photothermolysis pattern. Further studies are necessary to investigate different cooling methods and parameters when used in combinations with such devices, as this may affect the tissue temperature.

This study has several limitations. One is the inability to mimic the natural temperature gradient in the skin that results from the combination of skin surface cooling and dermal blood flow in a biological setting, which can have impact on the thermal damage pattern. However, we do not believe that this would have significant impact to our findings. Another limitation is that we only fully assessed the variability of epidermal MTZs, and did not investigate the diameter and depth of dermal MTZs, because this would require the three-dimensional reconstruction of each MTZ to evaluate the volumetric changes. Furthermore, the blinded investigator was advised not to incorporate overlapping MTZs into the evaluation, which might theoretically have led to the overestimation of the thermally injured surface area at higher skin temperatures, because the likelihood of an overlap of individual MTZs increases with the diameter of the MTZs.

In conclusion, there is a positive linear relationship between the epidermal MTZ diameter and skin temperature. This makes skin temperature during fractional photothermolysis an important variable factor. The use of simultaneous skin cooling increases patient comfort; however, as it also decreases MTZ size, it may interfere with treatment efficacy. The control of skin temperature is
necessary to provide a consistent outcome and to be able to compare treatments.

REFERENCES


