Histopathology of Laser Skin Resurfacing

Sharon Thomsen [a], Bonnie Baldwin[a], Eric Chi[b], Jeff Ellard[a] and Jon Schwartz[a]
[a]U. Texas M. D. Anderson Cancer Center, Houston, TX 77030
[b]Rice University, Houston, TX 77030)

ABSTRACT

Pulsed CO2 laser skin resurfacing is a purportedly "non-thermal" procedure enjoying wide application as a cosmetic treatment for skin wrinkles. Treatment success has been based on clinical assessments of skin smoothness. Skin lesions (1 cm^2) created by one, two or three superimposed CO2 laser passes were placed on the backs of 28 "Fuzzy" Harlan Sprague Dawley rats. The variable laser irradiation parameters included measured energies ranging from 112 to 387/pulse with pulse widths of 65 and 125 μs anda repetition rate of 8 Hz. The square, flat laser beam measured 3 mm^2 at the focal point. The lesions were collected from 0 to 10 days after treatment for qualitative and quantitative histopathology. Thermal damage and treatment effect tended to increase in severity and, to a lesser extent, depth with increased delivery parameters. In acute lesions, the vacuolated and fragmented, desiccated and thermally coagulated epidermis was partially removed exposing the underlying thermally coagulated dermal collagen and cells. Epidermal and dermal necrosis and slough occured between 24 to 72 hours after treatment. Epithelial regeneration originated from the adnexa and the lesion edges. Dermal fibrous scar formation began at 5 days below the regenerated epidermis and became more prominent at 7 and 10 days.

Key Words: laser skin resurfacing, thermal damage, wound healing

INTRODUCTION

Pulsed CO2 laser irradiation is a popular cosmetic treatment for facial skin wrinkles usually due to excessive sun exposure, aging and smoking. Clinically, the treatment includes one, two or three passes depending on the desired effect. One irradiation pass produces a light tan "dust" on the skin surface. The "dust" is wiped off and a second and even a third pass are applied to "shrink" and smooth the skin. The treated area is covered ususally with petroleum jelly and/or antibiotic ointments. Crusting occurs within a few days and the skin can become reddened but this can be covered with make-up. Clinical success is determined by the smoothness of the skin with demunition or disappearance of the wrinkles. Complications include prolonged skin reddening, scarring and hypo or hyper pigmentation. The purported advantages of laser irradiation over dermabration (mechanical removal of skin) and deep chemical peel are 1) the treated area doesn't bleed and 2) the treatment depth can be controlled.

Various mechanisms have been proposed for the treatment effect including cell and tissue vaporization, desiccation, extra cellular matrix contraction and photomechanical skin removal. Recent histopathological and temperature measurement studies suggest thermal changes including coagulation and ablation in treated skin extending into the reticular dermis. [1]

We performed in vivo, survival skin resurfacing experiments to test the following hypotheses: 1) the basic mechanisms of pulsed $CO^2$2 laser skin resurfacing are thermal leading to tissue necrosis 2) the necrotic epidermis and superficial dermis are organized and/or sloughed during healing, 3) the necrotic dermis is replaced by fibrous scar tissue and 4) epidermal regeneration originates from adnexal epithelium and residual epidermis at the lesion edges.

MATERIALS AND METHODS

Skin resurfacing lesions (1 cm^2) were placed on the midline backs of 28 anesthetized "Fuzzy" Harlan Sprague Dawley adult rats. These immunilogically competent, genetic mutant animals produce only fine under fur therefore do not require excessive depiliation. Their skin was prepared by shaving followed by gentle chemical depiliation. The lesions were created by sweeping the laser beam in a spiral beginning from the periphery to the center of the 1 cm^2 area which was demarcated by ink dots. The "dust" of the one pass lesions was not removed but the surfaces of the two pass and three pass lesions were wiped with saline soaked gauze pads then dried after the first pass. The irradiation times were about 3 to 4 seconds for each pass and the interavals between passes were about 15-20 seconds long. The laser hand piece was held perpendicular to the skin surface at the focal point of the focused beam. The handpiece was moved by the operator recapitulating recommended clinical application methods.

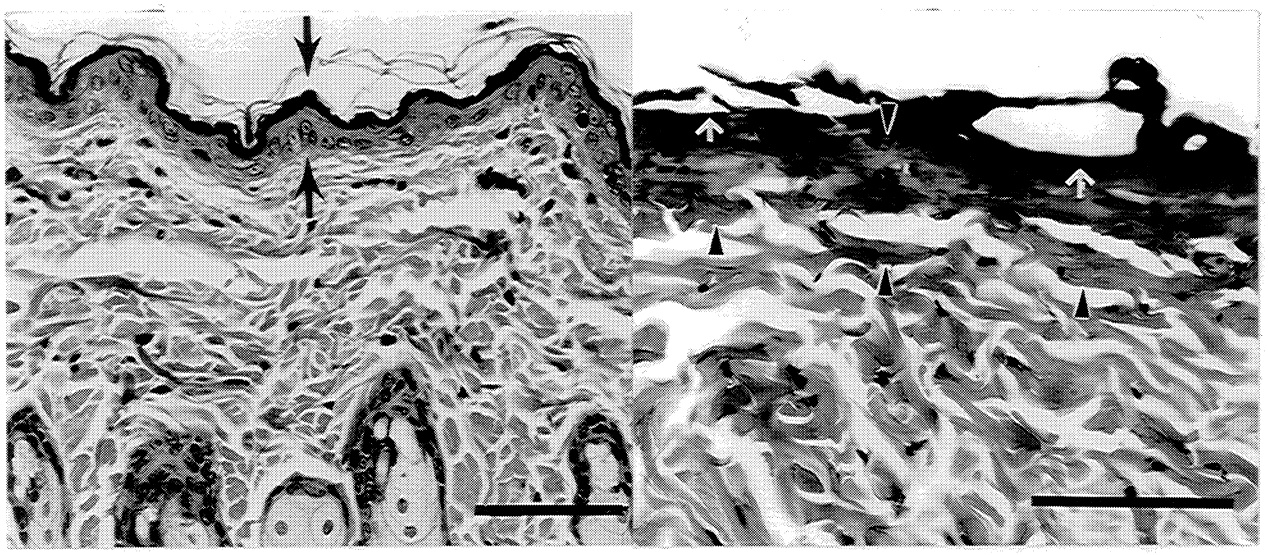
A commercial model of a TruPulse CO2 (λ = 1.064 μm) laser was used to make the lesions. The parameters were chosen initially guided by LED read-outs that indicated milliJoules (250 and 500 mJ) and pulse widths (65 and 125 μs). However, the actual delivered energy was calculated as mJ/pulse based on measurements of the beam power delivered through the hand piece using a power meter. Experience showed great variation of laser power especially with continued use over the day and at high LED joulemeter readings. The calculated delivered energies ranged in groups of 112-122 mJ/pulse (250 mJ/65 μs), 212 -244 mJ/pulse (250 mJ/125 μs), 188-256 mJ/pulse (500 mJ/65 μs) and 288-388 mJ/pulse (500 mJ/125 μs). The spot size was 3 mm^2 and the repitition rate was 8 Hz. The beam profile was flat (top-hat).

The lesions were photographed and collected at 0, 1, 2, 3, 5, 7 and 10 days after creation of the lesions. Petroleum jelly was applied to survival animals at 0, 1 and 2 days. No infections were encountered. On the day of harvest, the animals were anesthetized, then sacrificed and the pelts removed. They were fixed in 10% buffered formalin. Each lesion was sampled twice including the lesions and adjacent skin. Paraffin sections were stained with hematoxylin and eosin, trichrome and elastin stains. The sections were examined using diffuse white light and transmission polarized microscopy. Qualitative and quantitative histopathologic analyses included obsevations of acute and delayed epidermal and dermal effects and measurement of the depths of thermal coagulation of dermal collagens (boundary of birefringence changes) in the acute lesions, the thickness of dermal inflammation and necrosis (day 2) and the thickness of dermal scar tissue (day 10).

RESULTS

Acute Lesions: The epidermis was deformed due to two thermal mechanisms, 1) water-dominated effects including vacuolization and desiccation and 2) thermal coagulation of proteins.[2] The most prominent change was tissue vacuolization and fragmentation of the epidermis which was more severe as the energy dose increases. No ablation was identified, however, patches of dermis were exposed with fragmented loss of epidermis ("popcorn effect"). The epithelial cells were shrunken, spindled, disrupted and hyperchromatic: histologic features of thermal coagulation and desiccation. The underlying dermal collagen was coagulated. (Figure 1) The coagulation was patchy at the lower engery levels and pass numbers but formed a more uniform, continuous band of hyalinization and birefringence loss at higher energies and with multiple passes. (Figure 2)

Days 1 & 2 Lesions: The thermally damaged epidermis was necrotic but remained clinging to the dermal surfaces in most lesions. Epidermal regeneration was beginning with the new epidermis coming from the hair follicles (adnexa) and the residual epidermis at the periphery of the lesions. The epithelium of the



(a) Normal **(b) Acute, One Pass Lesion**

Figure 1: Normal and One Pass Lesion (237mJ/pulse. 125 μs pulse) (a) Normal "Fuzzy" rat skin is composed of a thin epidermis (arrows) and the thick collagen fibers of the underlying dermis. The elliptical groups of large foamy cells in the dermis are sebaceous glands. (b) The residual epidermis in the one pass lesion is a dark, thin layer of thermally coagulated and desiccated tissue (arrows) disrupted by vacuoles and fragmentation. An irregular band of hyalinized, thermally coagulated dermal collagen (arrow heads) lies below the epidermis. [Hematoxylin and eosin stain. Bar = 100 μm]

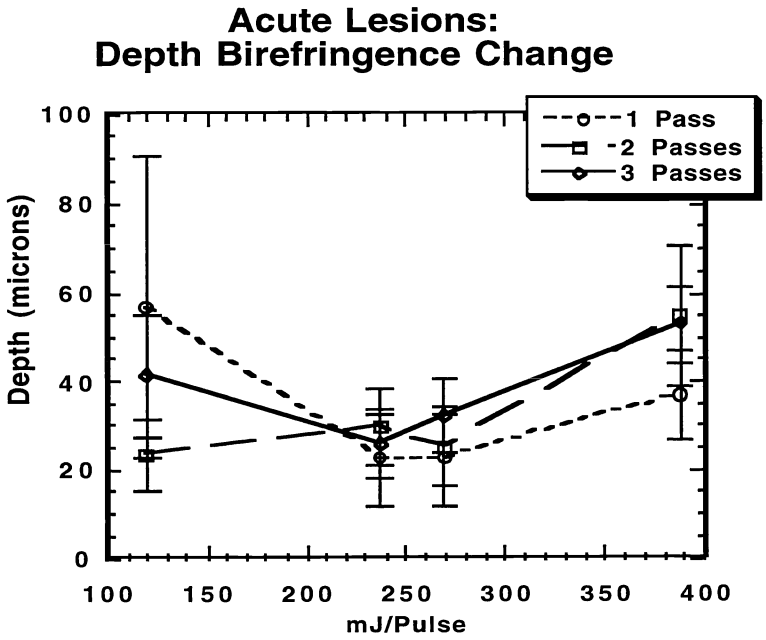


Figure 2

(a) **(b)**
Light Microscopy **Transmission Polarizing Microscopy**

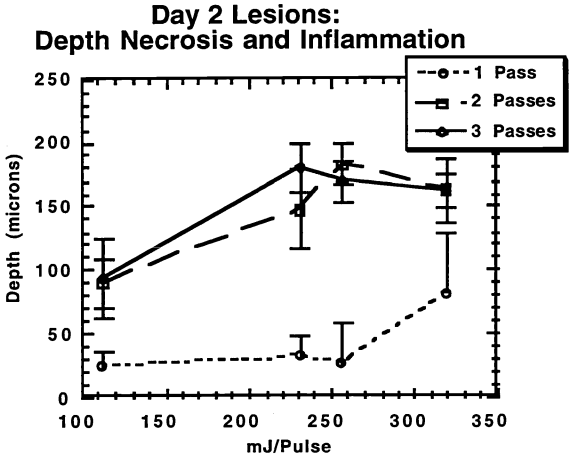
Figure 3: One Day Survival, Two Pass Lesion (244 mJ/ pulse. 125 μs pulse) (a) The lower border of the dense infiltrate of inflammatory cells (arrows) separates the overlying necrotic dermis from the viable dermis. The epidermis has sloughed in this lesion. (b) Same field as (a) The necrotic dermis includes the band of thermally coagulated collagen (birefringence loss, arrowheads), subjacent dermis and inflammation (arrows) [Hematoxylin and eosin stain. Bar = 100 μm]

hair follicles and their sebaceous glands was variably coagulated and necrotic with the damage extending more deeply in those lesions formed at higher energies. Focal infiltrates of inflammatory cells collected beneath the adherent epidermis in the one pass lesions. However, in the two and three pass lesions, relatively large areas of dermal necrosis weredemarcated by deeper bands of inflammatory infiltrates. The dermal necrosis encompasses not only the thermally coagulated dermal collagen but also a deeper layer of dermis in a pattern reminiscent of pan-tissue necrosis. (Figure 3) This necrosis and inflammation is maximum at two days. In the two and three pass lesions, the necrotic and inflammatory band depth increases with increasing energies but tends to plateau above 230 mJ/pulse. Although there are no significant differences between the depths of the two and three pass lesions, they are significantly larger than the one pass lesions. (Figure 4)

Day 3 Lesions: Eschar ("scab") formation which included the necrotic epidermis, adnexa and dermis was prominent in the two and three pass lesions but focal in the one pass lesions in which most of the epidermis had regenerated. The regenerated epidermis was about twice as thick as the normal epidermis and the skin surface tends to be flattened. The eschar clung to the skin surface; however, regenerating epidermis was found beneath the scab.

Days 5 & 7 Lesions: By this time, most of the eschar has been sloughed with a few fragnments still clinging to the surfaces of some of the two and three pass lesions. The epidermis is completely regenerated in the one pass lesions. By day 7, the epidermis of all lesions has completely regenerated but is now only slightly thicker that the normal epidermis. New small blood vessels (capillaries) and a few spindled cells resembling fibroblasts were first seen in the dermis just under the regenerated epidermis at 5 days and distinct bands of subepidermal fibrosis appeared at 7 days

Day 10 Lesions: The dermal band of scarring fibrosis was more prominent at 10 days with the band depth tending to be larger in the two and three pass lesions than the one pass lesions but not significantly so. (Figure 5) The fibrous band is composed of thin collagen fibers that lie parallel to the skin surface. (Figure 6)



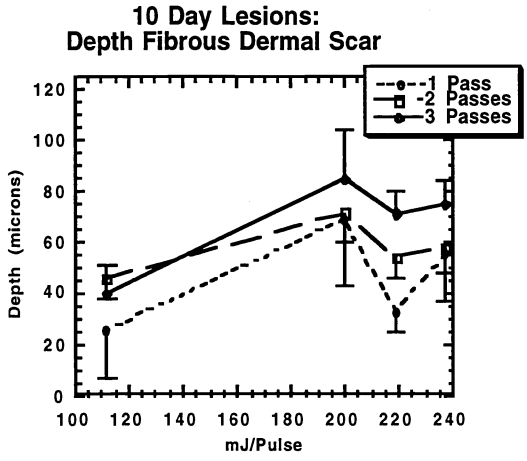


Figure 4

Figure 5

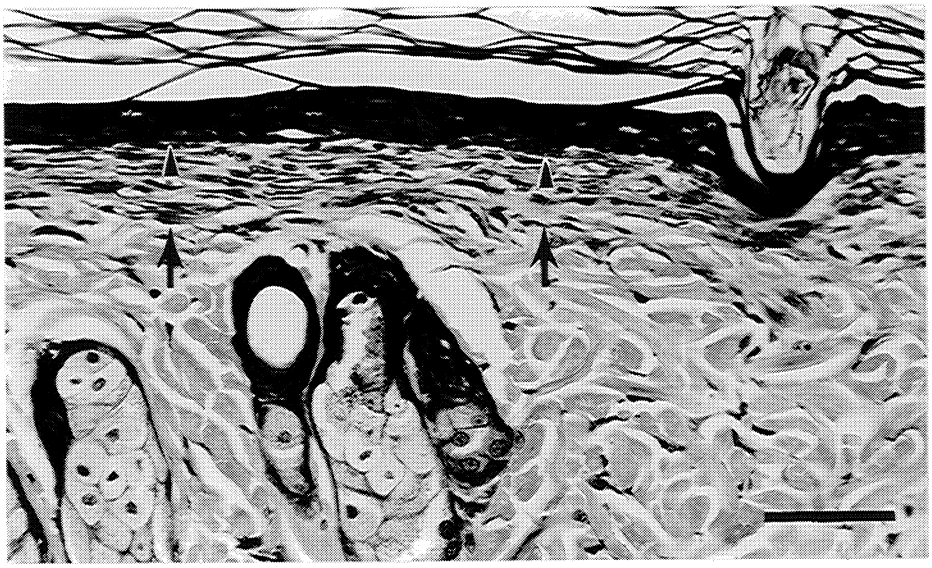


Figure 6: 10 Day Survival. Two Pass Lesion (237 mJ/pulse. 125 μs pulse). The collagen fibers and fibroblasts of the dermal fibrous scar (arrows) form a uniform band just below the regenerated epidermis (arrow heads). [Masson's trichrome stain. Bar = 100 μm]

Discussion

The basic mechanism of pulsed CO2 irradiation for skin resurfacing using the TruPulse laser is photothermal. The water of the epidermis is vapourized producing tissue desiccation and formation of intraepidermal steam vacuoles which seem to enlarge as the energy/pulse increases.[2] Rupture and fragmentation of the thin-walled vacuoles is more prominent in the two and three pass lesions. However, thermal coagulation of the epidermal cellular proteins results in a significant epidermal residuum thermally fused to the demal surface. Thermal coagulation of collagen is the dominant effect in the upper layers of the dermis. Hyperchromasia and spindling of epithelial cells of the hair shafts indicate lethal thermal damage that extends beyond the sharp, measurable boundaries of hyalinized collagen and birefringence change. It is possible that lethal cellular damage (fibroblasts and blood vessels) extends deeper into the dermis but that cannot be verified in the acute lesions using light microscopy techniques.

The ultimate depth of treatment effect is seen at 2 days when a measurable zone of dermal necrosis and an underlying layer of inflammation is seen. This necrosis is most uniform in the two and three pass lesions and is significantly thicker than the necrosis in the one pass lesions. The necrotic/inflammatory zone is considerably thicker than the zone of birefringe change. The necrosis is similar to necrosis secondary to blockage of blood flow (infarction) in tissues. These findings suggest that lethal thermal damage to dermal blood vessels occurs that leads to the delayed injury response of skin necrosis with multiple passes.

Epidermal regeneration is rapid in the "Fuzzy" rat and is especially rapid in the one pass lesions where spotty dermal coagulation was seen. However, the most important observation in the one pass lesions was that relatively little epithelium of the adnexa appeared to be thermally damaged. Therefore, unlike the two and three pass lesions, the living source of regenerating epidermis was closer to the surface in the one pass lesions and only a few areas of dermal necrosis interfered with the healing process.

Cicatricial dermal fibrous began after epidermal regeneration. The scar depth was uniform and tended to be slightly thicker in the two and three pass lesions than in the one pass lesions. The presence of significant fibrous scarring in the one pass lesions at 10 days suggests that the fibrosis is not necessarily related to the extent of dermal necrosis/inflammation seen at two days. It is possible that secretion of growth factors from the regenerating epidermis and other surviving tissues stimulates the scar formation as a late and secondary response to the thermal injury.[3] The fate of this scar cannot be determined from this study which included only 10 days survival.

These patterns and sequences of wound healing are characteristic of healing in superficial, open wounds due to a variety of causes in rats but are not necessarily characteristic of humans. Rat wounds heal rapidly, days faster than comparable wounds in humans. In addition, the anatomy and physiology of rat skin are considerablly different than those of humans since the rat does not have a well-formed papillary dermis or rich, subepidermal vascular plexus extending from the deeper dermis. The differences in blood distribution and supply could greatly influence the healing process. The depth of thermal effect may be modified by convective heat transfer by the rich human blood supply. Although the thickness of the rat dorsal skin approaches that of the human face skin around the eyes, the results of this study cannot be applied directly to the human situation because of the species differences.

The results of this study indicate that the CO2 irradiation effect extends beyond the 20 μm optical penetration depth of hydrated skin. The deeper dermal effects of the multiple passes may be a combination of deeper CO2 light penetration in the desiccated tissues as well as heat diffusion..

In conclusion, the cosmetic effects of TruPulse pulsed CO2 laser skin resurfacing in experimental rats are due to 1) lethal acute and delayed thermal injury of the epidermis and dermis, 2) slough of necrotic upper layers of the skin in multiple pass lesions, 3) epithelial regeneration and 4) delayed dermal fibrous scarring.