

Ultrastructural Changes Elicited by a Non-Ablative Wrinkle Reduction Laser

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Background and Objectives: Cosmeceuticals, chemical peels and collagen injections are used to rejuvenate skin, but none of these methods is effective or permanent. Recently, laser resurfacing has been found to be effective, but the incidence of side effects is relatively high. Two years ago, the non-ablative wrinkle reduction laser (N-Lite, ICN Photonics, UK) was developed, and there have been several reports about its clinical effectiveness. In this study, we have investigated ultrastructural changes elicited by exposure to the N-Lite laser.

Study Design/Materials and Methods: Eight adult volunteers were recruited for this study. They were treated with the N-Lite laser and 3-mm skin punch biopsies were obtained 3 hours, 1 day, 3 days, 1 week, 2 weeks, 4 weeks and 5 weeks after the laser exposure. These specimens were examined by electron microscopy.

Results: Three hours after the laser therapy, the capillaries showed endothelial cell edema with hemostasis and marked edema was observed around them. Neutrophils, monocytes and mast cells were observed in the extravascular dermis. These acute dermal inflammatory changes were observed until 1 week after the laser treatment. Two weeks after the laser treatment, the capillaries showed an almost normal structure, and dermal edema was not observed around them. New elastic fibers and collagen fibers had increased around the capillaries. Four weeks after the laser treatment, interstitial fibrosis was observed around the capillaries.

Conclusions: N-Lite laser irradiation leads to interstitial fibrosis, especially around the capillaries, 4 weeks after the laser irradiation. *Lasers Surg. Med.* 32:46–49, 2003.

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Key words: ultrastructure; collagen replenishment; N-Lite; wrinkle reduction

INTRODUCTION

Monochromatic laser light at 585 nm is known to induce selective collagen replenishment [1–3]. Using the suction blister method, irradiation with a 585 nm laser source induced more than an 80% increase in collagen within 72 hours of a single treatment.

The mechanism of collagen replenishment is considered to involve the microvasculature in the dermis. There were no clinical signs of skin damage, such as purpura, blister-

ing or other indications of vessel damage. However, a few histological reports using H&E staining found no dermal changes.

In this study, we have investigated the dermal changes elicited by the laser irradiation at the ultrastructural level.

MATERIALS AND METHODS

Laser Source

A 585-nm laser (N-Lite, ICN Photonics, UK) was used for this study. It emits pulses at a wavelength of 585 nm, with a spot size of 5-mm diameter.

Patients

Eight adult healthy volunteers (mean age 56.3 years, range 25–66 years) were recruited for this study; informed consent was obtained. All subjects were Japanese and had Fitzpatrick's type IV skin. Exclusion criteria for this study included chronic illness, cutaneous contact dermatitis, atopic dermatitis, photosensitivity, history of scar formation, vascular disease, stasis dermatitis, history of poor wound healing or any cutaneous illness.

Procedure

An area was chosen on each patient's fibula. Each area was treated with the N-Lite laser at 3 J/cm², with a pulse duration of 350 microseconds. Three millimetre skin punch biopsies were obtained from each subject 3 hours after the irradiation, as well as 1 day, 3 days, 1 week, 2 weeks, 4 weeks and 5 weeks later. They were fixed with 2.5% glutaraldehyde and were post-fixed with 1% osmium tetroxide. The tissue samples were dehydrated through a graded alcohol series and were embedded in Epon 812. Ultrathin sections were cut on an ultracut N ultramicrotome (Ultracut UCP, Reica, Germany) with a diamond knife and were stained with uranyl acetate and lead citrate. The sections were examined in an electron microscope (75 kV, Hitachi H-7500; Hitachi, Tokyo, Japan) [4]. This study was approved by the Ethics Committee of the Japan Equestrian Federation for Sports Medical Research.

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Accepted 9 August 2002

Published online in Wiley InterScience

(www.interscience.wiley.com).

DOI 10.1002/lsm.10119

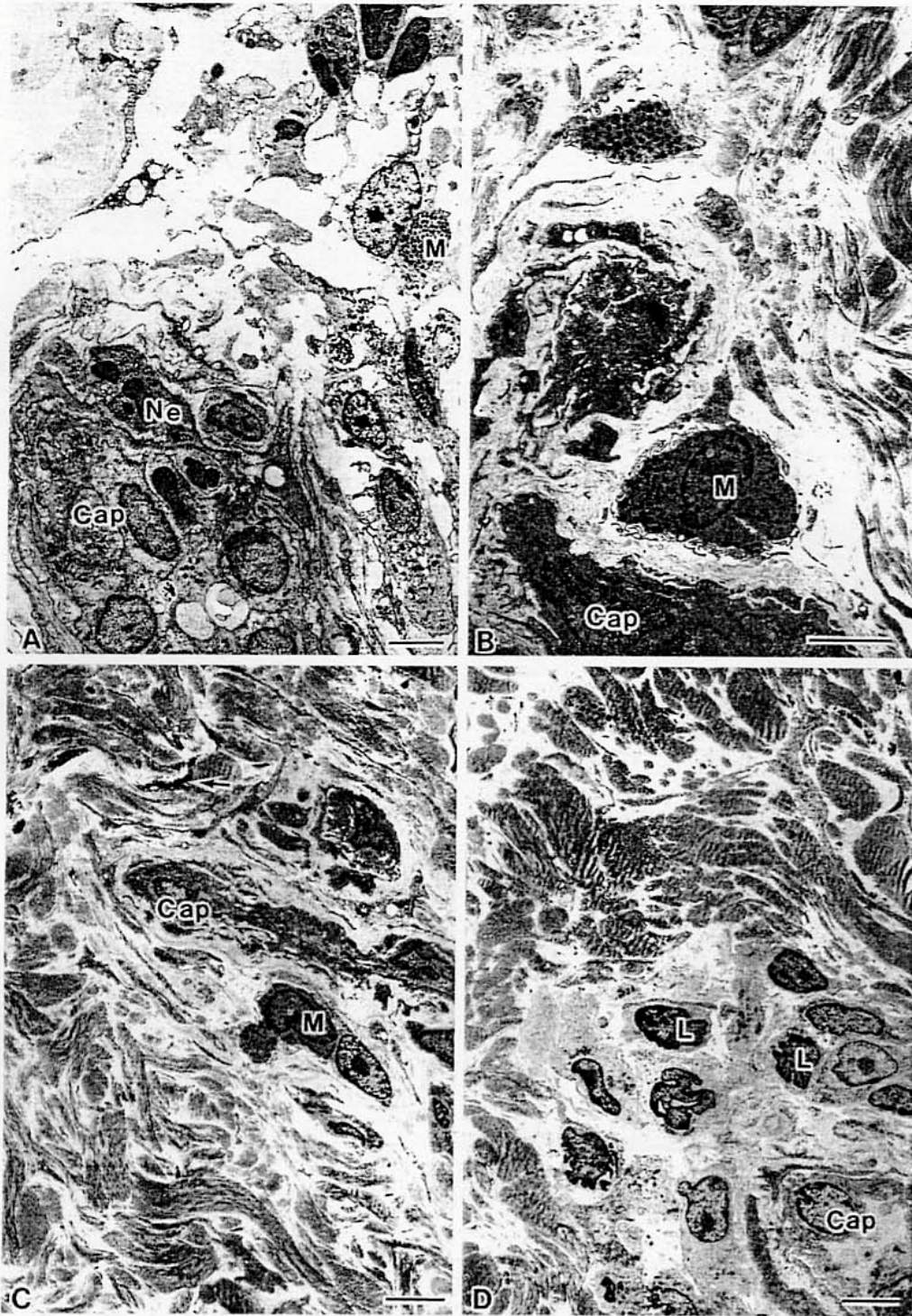


Fig. 1. **A:** Three hours after the laser therapy, the capillaries (Cap) showed endothelial cell edema with hemostasis, and endothelial cells were round and swollen. The basement membranes of the capillaries were thick and had several lamellar structures. Marked edema was observed around the capillaries. Neutrophils (Ne) and mast cells (M) were observed in the extravascular dermis. **B:** Two weeks after the laser therapy, the capillaries (Cap) showed an almost normal structure, and

dermal edema was not observed around them. Mast cells (M) were observed around the capillaries. **C:** Two weeks after the laser therapy, new elastic fibers (\uparrow) and collagen fibers had increased around the capillaries. The number of M increased from 1 to 2 weeks after the laser therapy. **D:** Five weeks after the laser therapy, interstitial fibrosis was observed around the Cap. Many lymphocytes (L) and fibroblasts were also observed.

RESULTS

The histological findings in the biopsy specimen did not differ significantly among the patients. For this reason, the results obtained in individual patients are not presented separately. Three hours after the laser therapy (Fig. 1A), the capillaries showed endothelial cell edema with hemostasis, and the shape of endothelial cells were round and swollen. The basement membranes of the capillaries were thick and had several lamellar structures. Marked edema was observed around the capillaries. Neutrophils, monocytes and mast cells were observed in the extravascular dermis.

These dermal acute inflammatory changes were observed also at 1 week after the laser treatment.

Two weeks after the laser treatment (Fig. 1B), the capillaries showed an almost normal structure. Dermal edema was not observed around the capillaries, and new elastic fibers and collagen fibers were increased around them (Fig. 1C).

Four weeks after the laser treatment, interstitial fibrosis was observed around the capillaries. Many lymphocytes and fibroblasts were also observed. The numbers of these lymphocytes increased further at 5 weeks after the laser treatment (Fig. 1D).

Table 1 summarizes the dermal changes after the laser therapy.

DISCUSSION

Five hundred eighty-five nanometer lasers are used for the therapy of vascular lesions because of their high absorption by methohemoglobin [5-7]. This study shows that treatment with a 585-nm N-Lite laser elicits marked edema of endothelial cells with hemostasis and dermal connective tissue. This edema was observed at all stages of this therapy.

The influence on capillaries seemed much milder compared with other 585-nm lasers, since histologically, total capillary damage and/or thrombi in the capillaries were observed following treatment with other 585-nm lasers [7]. In this study, erythrocytes were intact and endothelial cell

necrosis was not observed. One reason for this may be the difference in the fluence. Usually, 585-nm lasers are used around 6-7 J/cm² to treat vascular lesions [6], while the N-Lite laser is used at 2.5-3 J/cm² [2,3]. Another reason may be the wave shape pattern of the N-Lite laser, which can reach peak fluence rapidly in each pulse. This may decrease the damage of the whole capillary because the N-Lite laser does not have enough thermal relaxation time to damage the whole capillary.

Zelickson et al. [1] showed new collagen formation 12 weeks after treatment with a 585-nm pulsed dye laser using electron microscopy. We reported elaunin fibres in the dermis 3 days after the N-Lite laser treatment. Elaunin fibers are regarded as immature elastic fibers [8].

In this study, new collagen and elastic fiber formation [8] was detected 2 weeks after the laser treatment. This is a much more rapid appearance of new collagen formation compared with the study of Zelickson et al. We used just 2.5-3 J/cm², while Zelickson et al. used 6-6.5 J/cm². The higher fluence may damage the dermis more, and it may take longer for the tissue to repair.

New collagen formation was readily observed within 4 weeks of the laser therapy. Clinically, wrinkle improvement is also observed from 4 weeks after the therapy, and this study may reflect the clinical effects from histological observations.

Neutrophil and monocyte dominant infiltrates were observed from 3 hours to 1 day after the laser therapy, and this may be regarded as an early inflammatory change. We found marked mast cell migration around the capillaries. In this study, mast cell migration was seen from 3 hours to 4 weeks after the laser therapy, the most obvious being at 1 and 2 weeks after the therapy.

The granules of these mast cells were all of the granular type, and crystal type or scroll type granules were never observed. Usually, granules of cutaneous connective mast cells are of the crystal or the scroll type [9,10]. Mast cell granules change to the granular type from the crystal or scroll type during degranulation, and mast cell granules are of a ghost type at the full inflammation stage [9,10].

TABLE 1. Dermal Changes After the Laser Therapy

	3 hours	1 day	3 days	1 week	2 weeks	4 weeks	5 weeks
Changes of capillaries							
Swelling of the nuclear cells of capillaries	+	+	+	+	-	-	-
Hemostasis	+	+	+	-	-	-	-
Changes of interstitial structure around capillaries							
Interstitial edema	+	+	+	±	-	-	-
Interstitial fibrosis	-	-	-	-	±	+	+
Inflammatory cells							
Neutrophils	+	+	-	-	-	-	-
Monocytes	+	+	-	-	-	-	-
Mast cells	+	+	+	++	++	±	-
Lymphocytes	+	-	-	-	-	+	++

+: Moderate change or moderate increase of number.

++: Marked change or marked increase of number.

±: Mild changes or mild increase of number.

Granular type granules are not usually found in normal skin, but are only found in fetal skin or at stages of cutaneous illness [9]. In this study, ghost type granules were not observed, and thus the N-Lite laser may induce only very mild inflammation.

Mast cells are considered to correlate with collagen production or with new capillary formation [11,12]. Cytokines and growth factors are considered to be released from those granules [9,10], and those cytokines or growth factors should eventually stimulate new collagen replenishment [11,12]. In this study, the increased number of mast cells at 1 and 2 weeks after the laser therapy suggests that the role of these mast cells is not for inflammatory change but for new dermal replenishment.

Lymphocytes had increased at 4 and 5 weeks after the laser therapy. The role of these lymphocytes is not yet clear, but lymphocytes are also considered to play an important role in stimulating epithelioid cells and fibroblasts [13].

In this study, we made investigation by using fibula skin. N-Lite is mostly used for face skin. But ultrastructurally collagen structure and capillary structure are almost same between leg skin and face skin [14–16].

During this study, focal marked edema was observed in the acute stage, but interstitial arrangement generally showed a normal structure. These observations may reflect a clinically normal reaction without any purpura or irreversible damage from the laser exposure [2,3]. In the wound healing process, dermal edema is considered to play an important role in new dermal formation due to inflammatory cell migration [13]. The edema found in this study may also help to produce new collagen arrangement.

N-Lite may induce mild inflammation, and collagen replenishment may reflect the fibrotic dermis during mild wound healing process after mild inflammation.

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