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Histologic and photonic evaluation of a pulsed Nd:YAG laser for ablation of subcutaneous adipose tissue

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Although various lasers are available, few of them are applicable in liposculpture. Laser interaction with fat tissue has not also been well documented. The aim of our study was to gather basic data on laser absorption in fat tissue and to analyze the relationship between laser energy and lipolysis for development of a more effective laser system.

The transmittance rate in human fat specimens was measured by a spectrophotometer to determine the optimum wavelength. The absorption coefficient was used to evaluate laser absorption at a wavelength of 1064 nm. Areas of heat degeneration and evaporation were measured by scanning electron microscopy. The relation between laser energy and the areas was analyzed statistically among low-power and high-power groups and controls. Energy dispersion at the fiber tip was investigated and analyzed statistically using the far field pattern.

A graph of the absorption rate at wavelengths from 400 to 2400 nm showed a peak near 1700 nm and increases at wavelengths over 2000 nm. The formula gave as an absorption coefficient of 0.4 cm^{-1}, and involvement of the photo-acoustic effect and non-linear effect with short-pulse and high-peak energy was suggested. Findings of tissue evaporation, destruction, heat coagulation, and rupture of cell membrane were more frequently seen in irradiated specimens than in controls in scanning electron microscopy. The destroyed area in the low-power irradiated groups was significantly larger than that of controls in the statistical analysis. The affected area in the high-power irradiated groups was significantly larger than that of low-power specimens. Energy was concentrated at the tip with laser coherency. Energy at the oblique-cut tip was statistically lower than that at the normal tip, revealing that durability and maintenance of the fiber tip is essential to maintain energy levels in clinical practice.

This study is the first to demonstrate the histologic and photonic relationship of energy absorption and lipolysis using a pulsed Nd:YAG laser. The results will be useful for research and development of a more effective laser system for liposculpture.

Key words: laser, adipocyte, histology, photonics

BACKGROUND AND INTRODUCTION

Various complications associated with liposculpture using metal cannulas with negative pressure and excisional lipectomy have been reported [1-3]. A pulsed Nd:YAG laser was recently reported to be potentially applicable for lipolysis [4,5]. Although various lasers are available, no other laser is practical for use in liposculpture. However, histologic evidence and a photonic study of laser absorption in human fat tissue have not been reported except by the authors [6].

The authors have studied and developed medical lasers in cooperation with the School of Engineering and laser companies since the Department of Plastic Surgery of Tokai University was established. In recent studies on pulsed lasers, we reported subcutaneous apocrine gland ablation [7], endolaser venous ablation [8] and a histologic study of laser lypolysis [6]. Laser interaction with fat tissue has not been well documented in the field of laser medicine to date. We studied laser absorption in human fat tissue, histologic findings of laser lypolisis, and the relationship between irradiated energy and degree of fat destruction. The aim of our study was to gather and analyze basic data on laser absorption in fat tissue for development of a more effective laser system for liposculpture.

MATERIALS AND METHODS

The tissues were taken from excised excess parts of skin flaps containing a sufficient amount of subcutaneous fat generated from plastic surgery operations. All three subjects gave informed consent based on the Tokai University Hospital Institutional Review Board-based protocol. Eighteen tissue samples of $2 \times 2 \times 1$ cm tissue were used. The tissues were irradiated or cannulated immediately after excision.

We used a pulsed Nd:YAG laser (Smartlipo, DEKA, Italy), a variable-hertz, variable-Joule, 1064-nanometer laser system. The laser light is conveyed through micro-cannulas with a diameter of 1 mm into which an optical fiber of 300 μm is inserted (Fig. 1). A 100 μsec pulsed laser at 40 Hz and 150 mJ was used for the high-power group (n = 6), and a 100 mJ laser for the low-power group (n = 6). The cannula was inserted into the target layer of the subcutaneous fat. The laser was applied to the tissue with an extracting motion of two cm/sec. The laser was applied to the target layers for one sec each with repeated cannulation, and the to-

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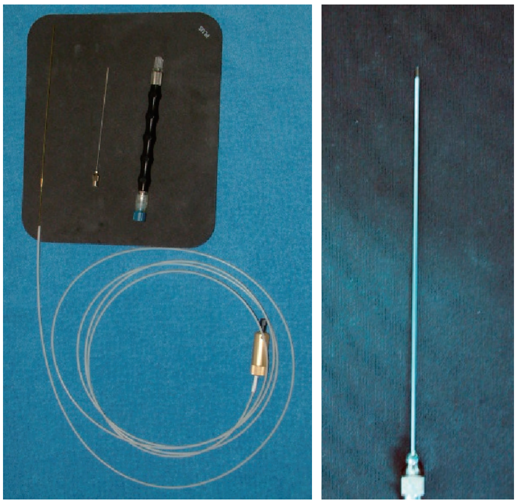

Fig. 1 The laser is conveyed through microcannulas with a diameter of one mm into which an optical fiber of 300 μm is inserted.

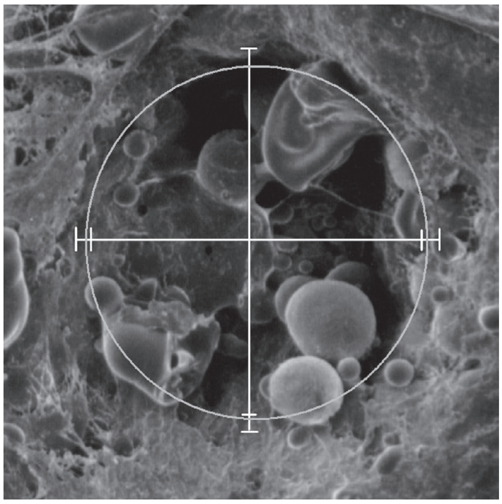

Fig. 3 Hollow areas due to fat destruction and evaporation were measured by scanning electron microscopy. Approximation to circles and ellipses was used for calculation because specimens were cut perpendicularly to the direction of cannulation and irradiation.

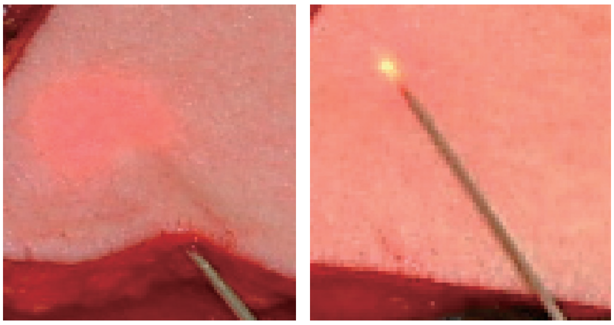

Fig. 2 Laser irradiation procedure. The cannula was inserted into the target layer of the subcutaneous fat. The laser was applied to the tissue with an extracting motion of two cm/sec.

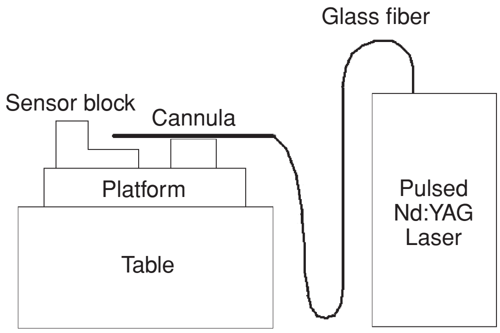

Fig. 4 System to measure the far field pattern of the laser. Energy setting of a 100 μsec pulsed laser at 30 Hz and 30 mJ was used for measurement.

tal duration of exposure was three sec for each sample. The cannulation and irradiation procedures are shown in Fig. 2. In the control group (n = 6), samples were processed in the same manner without irradiation. In all groups, suction was not applied to the cannula during passage through the tissue. Tissues were fixed immediately after irradiation or cannulation in glutaraldehyde and osmium for scanning electron microscopy.

The transmittance rate in the fat specimens was measured by a spectrophotometer to determine the optimum wavelength for laser lipolysis. The absorption coefficient was used to evaluate laser absorption at a wavelength of 1064 nm. According to the data, the absorption coefficient was calculated as

$$\alpha = \frac{1}{d_2 - d_1} In \frac{T(d_1)}{T(d_2)}$$

Where d and T(d) are the tissue thickness and the transmittance ($d_2 > d_1$), respectively. Reflection and scattering were not taken into consideration.

Hollow areas due to fat destruction and evaporation were measured by scanning electron microscopy (Fig. 3). Approximation to circles and ellipses was used for calculation because specimens were cut perpendicularly to the direction of cannulation and irradiation.

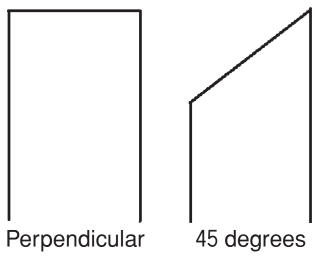

Fig. 5 Conditions of the fiber tip angle at zero and 45 degree were compared in the measurement of the far field pattern.

The relation between laser energy and the areas was analyzed statistically among low-power groups, high-power groups and controls.

Energy dispersion due to dullness and angles of the fiber tip was investigated using the far field pattern with Gaussian fitting. Data between the zero angle and 45 degree angle were analyzed statistically to evaluate the importance of maintenance the fiber tip in clinical practice. The far field pattern of the laser was estimated by the Division of Applied Sciences and Photonics, Tokai University School of Engineering (Fig. 4). A 100 μsec pulsed laser at 30 Hz and 30 mJ was used. Conditions of the fiber tip angle at zero and 45 degrees were compared (Fig. 5).

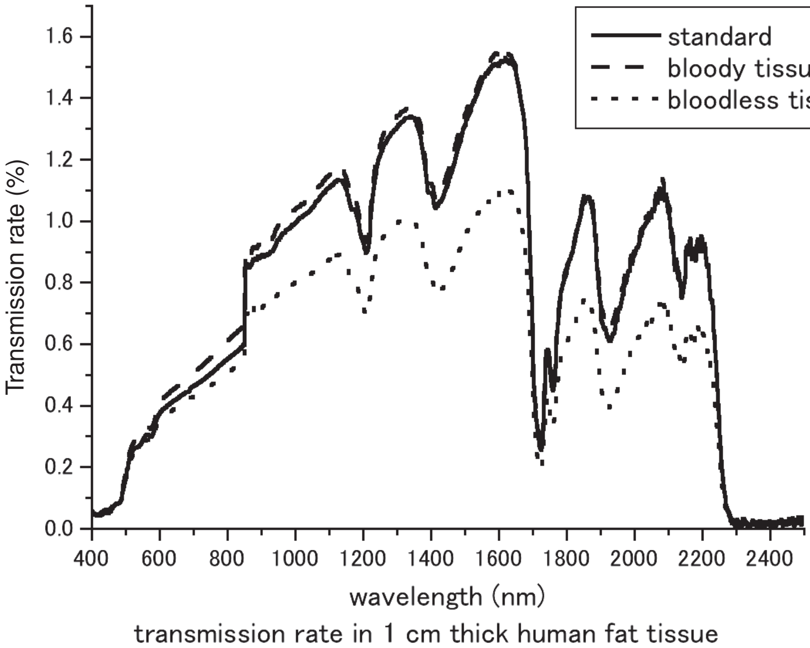

Fig. 6 Transmission rate of human fat at wavelength of 400 to 2400 nm.

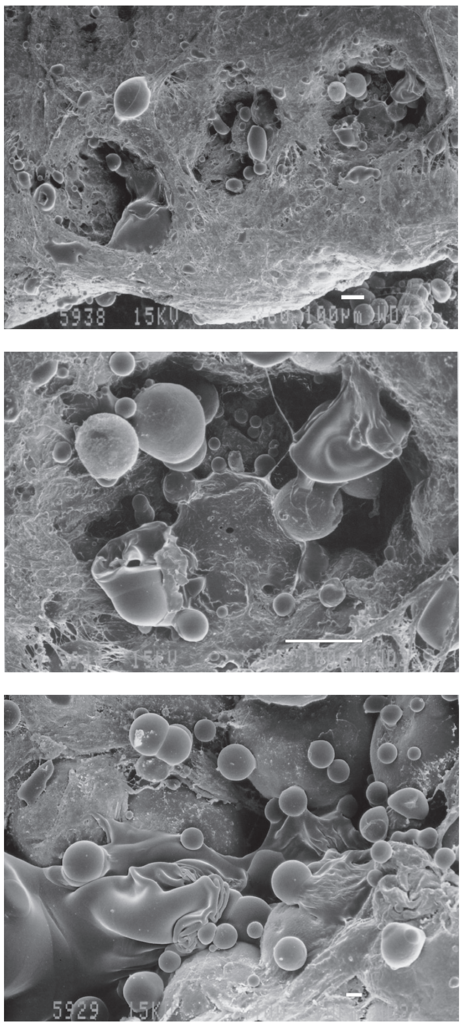

Fig. 7 Scanning microscopy of human specimens after laser irradiation showed destructive changes. Hollows due to irradiation were seen (*above*). Degenerated cell membrane and dispersed lipids were apparent (*center and below*). Scale bar = *above* and *center* 100 μm, *below* 10 μm. (Original magnification, *above* ×60, *center* ×200, *below* ×400)

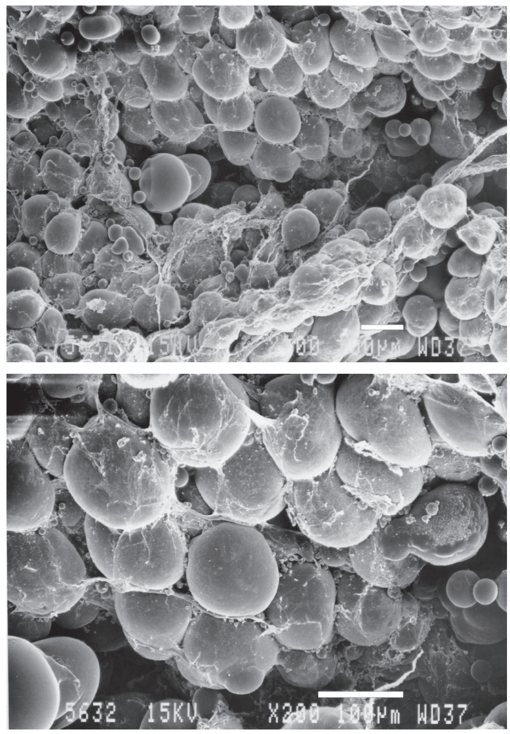

Fig. 8 Scanning electron microscopy of human specimens after cannulation without laser irradiation (control). No major structural changes were observed in adipocytes. Scale bar = 100 μm. (original magnification, *above* ×100, *below* ×200)

RESULTS

A graph of the absorption rate at wavelength from 400 to 2400 nm showed a peak at a wavelength near 1700 nm and an increase at over 2000 nm (Fig. 6). The formula gave 0.4 cm^{-1} as the absorption coefficient.

Findings of tissue evaporation, destruction, heat coagulation, and rupture of the cell membrane were more frequently seen in irradiated specimens than in controls in scanning electron microscopy (Figs. 7 and 8).

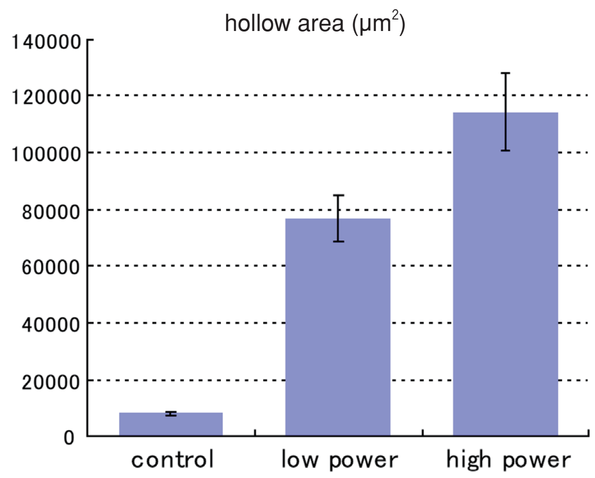

Fig. 9 Destroyed areas of low-power irradiated groups (76725 $\mu m^2 \pm 8315$) was significantly larger than those of controls (8055 $\mu m^2 \pm 480$) in statistical analysis (Student t-test, $p < 0.00001$). Affected areas of high-power irradiated groups (114283 $\mu m^2 \pm$ 13637) was significantly larger than those of low-power specimens (Student t-test, $p < 0.001$).

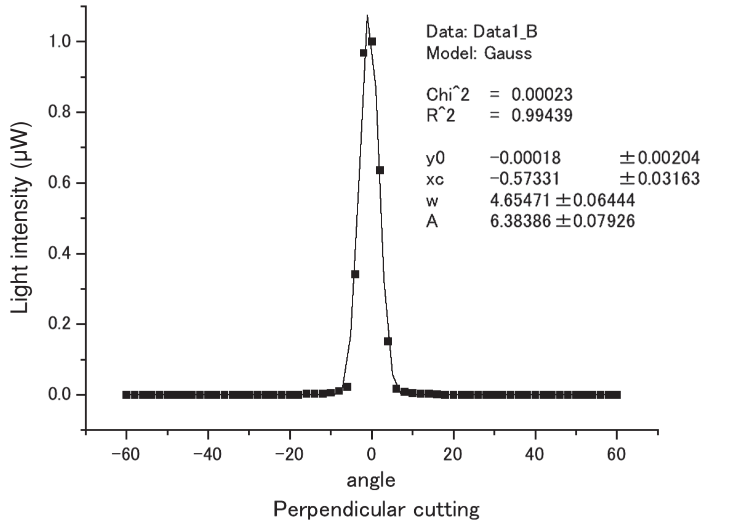

Fig. 10 Far field pattern of the pulsed Nd:YAG laser through the fiber tip with a zero degree angle. Discharged laser energy was sharply peaked and concentrated around the tip.

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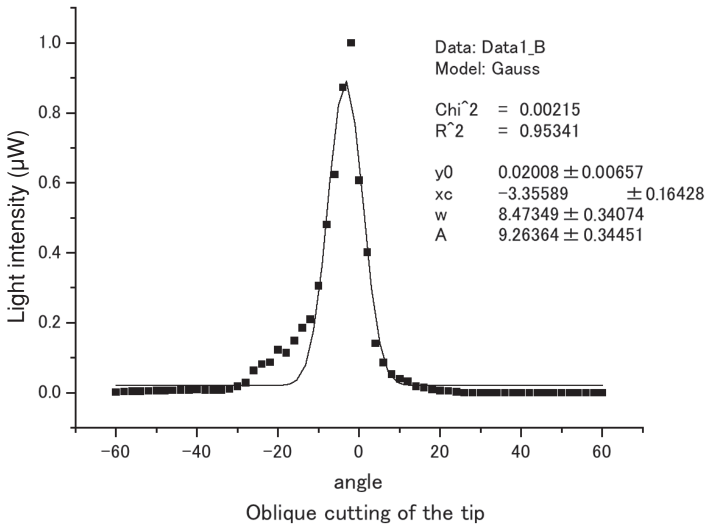

Fig. 11 Far field pattern of the pulsed Nd:YAG laser through the fiber tip with a 45 degree angle. Peak formation was not sharp at the base and some energy leakage was observed.

DISCUSSION

A variety of surgical and medical interventions have been reported to assist liposculpture including ultrasound, vibration, carbon dioxide injection and mesotherapy for prevention of associated complications with traditional liposuction and lipectomy [1-3, 9, 10]. The search continues to reduce downtime, operator effort and bleeding, and to achieve skin tightening, fine sculpture, and treatment of fibrous or reoperative areas.

Laser liposculpture is a new technique, which is still under development, and there is still no laser in common use for lypolysis except the laser we studied. Possibility of laser-assisted lipoplasty was first reported in 1992 by Apfelberg [11]. Apfelberg reported laser-assisted liposuction with the YAG laser beam enclosed in a cannula, but clear benefits over standard liposuction could not be demonstrated, and the system was not used in practise [12, 13]. Ultrapulse CO_2 laser application to vaporize subcutaneous fat was reported by Cook *et al.* [14] and Park *et al.* [15]. Instead of good tightening, they needed to incise and dissect the skin for laser exposure because of the large handpiece. The Er:YAG laser has been reported to be effective for facial rejuvenation with shorter downtime, but no reports on lipoplasty with the Er:YAG laser have been published. Low-level laser therapy has been enthusiastically reported as an adjunct to clinical aspirative lipoplasty by Neira *et al.* [16, 17]. They stated that 99 percent of the fat was released from the adipocyte after 6 min of 635 nm, 10 mW diode laser exposure. They also reported 700 cases treated with external low-level laser-assisted lipoplasty. However, in contrast, Brown *et al.* reported that no adipocyte structural differences were observed between low-level laser therapy and non-irradiated samples in their studies using the same methodology as that of Neira *et al.* [18].

According to the data from this study, a laser with a longer wavelength than 1064 nm should achieve effective lipolysis with lower output energy. The CO_2 laser and Er:YAG laser both have longer wavelengths.

However, the CO_2 laser is not suitable for stable irradiation through a thin cannula and Er:YAG laser presents the problem of bleeding. Diode laser with a near 1700 nm wavelength is a possible candidate for development of a stable and low-cost system although the laser has lower tissue permeability than the Nd: YAG laser, but the development of a high power system generating a short pulse with a high peak is a problem with diode lasers.

Laser lipoplasty with a pulsed Nd:YAG laser has been reported to be widely used in Europe and Latin America [4, 5], where the possibility of laser lipoplasty without liposuction was also reported. The system has recently been introduced in Japan and the United States. However, English references are few and there are no reports of controlled studies or evaluation with scanning electron microscopy. The authors reported a histologic evaluation of this laser [6].

In laser absorption into molecules in fat tissue, energy seems to be absorbed by carboxyl groups and phospholipids for molecular excitation and oscillation. However, photothermolysis dependent on wavelength is not sufficient to describe laser lipolysis. Based on data on the absorption coefficient, the photo-acoustic effect and non-linear effect with short-pulse and high-peak energy are assumed to be involved. The photo-acoustic effect of the laser is also involved in morphology of adipose destruction, dispersed fat lipids, and coagulated surrounding tissue. The laser shockwave appears to be effective in removing fat tissue. Shockwaves are generated by pulsed lasers with short duration and high peak power. The high-density energy in short pulses has an explosive effect on atoms within the target tissue. Electrons are inelastically scattered from the atoms and form a plasma shield that helps to screen deeper structures from the beam. After the pulse, released electrons are recaptured, giving off the energy that they gained from the photon pulse [19]. Although Kuwahara *et al.* discussed rupture of fat cells using laser-generated ultra short stress waves [20], no basic investigations exploring optimal wave-lengths or pulse-lengths for fat destruction have been reported.

This study also revealed that durability and maintenance of the fiber tip are essential to maintain energy level in clinical practice. According to the far field patterns, laser energy was highly concentrated at the tip. However, energy leakage was observed with oblique cutting of the edge. Cutting of the tip edge is often performed by scrub nurses in the operative theater, and it is important to train them in proper cutting.

In conclusion, this study is the first to demonstrate the histologic and photonic relationship of energy absorption and lipolysis using a pulsed Nd:YAG laser. The results will be useful for research and development of a more effective laser system for liposculpture.

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