Comprehensive histologic analysis of interstitial lipolysis with the 1444 nm wavelength during a 3-month follow-up

**So Dug Lim[1], Jong-In Youn[2], Won-Serk Kim[3], Il-Hwan Kim[4],
Tae Sook Hwang[1], Hye Seung Han[1] and Un-Cheol Yeo[3,5]**

[1]Department of Pathology, Konkuk University School of Medicine and Konkuk University Medical Center, [2]Department of Biomedical Engineering, Catholic University of Daegu and [3]Department of Dermatology, School of Medicine Sungkyunkwan University, [4]Department of Dermatology College of Medicine Korea University and [5]S&U Dermatologic clinic, Seoul, Republic of Korea

Summary. A number of near-infrared wavelengths have been proposed and studied for laser lipolysis, but the histologic evaluation of tissue response to laser lipolysis during long-term follow-up has been lacking. A 1444 nm Nd:YAG laser with better absorption in both fat and water has recently attracted attention. The present study was designed to investigate the comprehensive histopathology of 1444 nm Nd:YAG laser-assisted lipolysis at different energy levels during a 3-month follow-up. Laser lipolysis was performed on porcine fat tissue *in vivo* using a 1444 nm Nd:YAG laser (AccuSculpt®, Lutronic Corporation, Ilsan, Republic of Korea) and the total energies delivered interstitially to 10×10 cm^2 areas were 750 J, 1500 J, 2250 J, 3000 J, 3750 J, 4500 J, and 5250 J. Biopsy samples were taken and histologically analyzed immediately after biopsy and at 1, 2, 4, and 12 weeks postoperatively. With a fluence setting above 3000J/100 cm^2, inflammation was severe and remained by the 3-month follow-up, resulting in severe scarring of the fat tissue. Below this energy level, mild lobular inflammation in the early phase biopsy had resolved with no scarring by the 3-month follow-up. No histologic changes in the epidermis or dermal connective tissue were present. This study suggested that controlling the energy level is important for clinical applications of laser lipolysis with no significant complications.

Key words: Lipolysis, Laser, 1444 nm Nd:YAG, Histology

Introduction

A variety of surgical modalities has been used to reduce subcutaneous fat (Badin et al., 2002) and laser lipoplasty with pulsed neodymium, yttrium, aluminum, garnet (Nd:YAG) laser, also called interstitial lipolysis has recently been introduced to remove local fat and to use for skin tightening (Badin et al., 2005; Ichikawa et al., 2005; Goldman, 2006; Kim and Geronemus, 2006). A number of near-infrared wavelengths have been proposed and studied for laser lipolysis (Goldman, 2006; Kim and Geronemus, 2006; Mordon et al., 2007; Khoury et al., 2008; O'Dey et al., 2008). Currently, the 1064 nm wavelength is used most frequently and it is well known that pulsed 1064 nm Nd:YAG lasers effectively destroy fat cells (Mordon et al., 2007; Khoury et al., 2008; O'Dey et al., 2008). There are many reports of fat cell lysis following this procedure and histologic studies have focused only on the immediate postoperative period (Badin et al., 2005; Ichikawa et al., 2005; Goldman, 2006; Kim and Geronemus, 2006; Mordon et al., 2007; Khoury et al., 2008; O'Dey et al., 2008). Aside from examnining fat lysis on the immediate biopsy, however, no histopathologic assessment of laser lipolysis with long-term follow-up has been reported regarding the healing of injured fat tissue without significant complications.

Recently, a new line of the Nd:YAG laser at 1444 nm has attracted a great deal of attention as a potentially effective laser lipolysis wavelength (Roggan et al., 1999; Tark et al., 2009). As the absorption coefficients for fat, and especially water, at $\lambda=1444$ nm are much higher than those at 1064 nm and 1320 nm wavelengths which are currently the wavelengths of choice for laser lipolysis, fat tissue can be lysed more efficiently at 1444

nm than at other wavelengths and it is well localized to irradiated tissues that contain water (Roggan et al., 1999; Tark et al., 2009).

Tark et al. (2009) reported that the 1444 nm Nd:YAG laser showed a greater lipolytic effect compared to the 1064 nm Nd:YAG laser in *in vivo* mini-pig and *in vitro* human fat experiments. Although they observed the effective lipolyic features of the 1444 nm Nd:YAG laser lipolysis and noted its advantages over the 1064 nm Nd:YAG laser, they evaluated only the histology of the injured fat tissue using one energy level at the one month follow-up and observed serious complications, such as burns. They suggested that additional investigation is necessary due to the complications; hence, *in vivo* studies with a long term follow-up on how tissue repair occurs, how long inflammation persists, and the relationship between inflammation and energy levels remain to be investigated for safe use of the 1444 nm Nd:YAG laser on human patients.

We designed the present study to investigate the dose-ranging repair process of the 1444 nm Nd:YAG laser lipolysis by histopathologic analysis over a 12 week follow-up. We also evaluated the optimum dose range to achieve efficient lipolysis without persistent damage and scarring to the overlying and target tissues in terms of the safety guidelines for clinical application.

Materials and methods

Two female SPF Micro-pigs®, 12 months old and weighing 45 kg and 65 kg, were used in this *in vivo* study. The pigs were given professional care and their health was monitored by qualified veterinarians at a facility (PWG Genetics Korea, Ltd., Pyungtaek, Gyunggido, Republic of Korea) accredited by the Association for Assessment and Accreditation Laboratory Animal Care (AAALAC). The Micro-pigs were sedated with isoflurane inhalation, and hair within the test sites was shaved prior to laser treatment. The Micro-pigs were sedated with isoflurane inhalation, and hair within the test sites was shaved prior to laser treatment. The skin was superficially sutured on each corner of the 10x10 cm grids to enable their detection after treatment during follow-up, with six test sites on each side of the back of the first Micro-pig, and eight test sites on each side of the back of the second Micro-pig, giving a total of 14 test sites with two sites for each energy level (Fig. 1). The grids were at least 5 cm apart from each other. Tumescent saline solution was infiltrated prior to laser lipolysis. No cooling or suction was applied after laser treatment.

A 1444 nm Nd:YAG laser (AccuSculpt®, Lutronic Corporation, Ilsan, Republic of Korea) was used to perform the laser lipolysis. The 1444 nm laser was used at 8 W power, 100 μs pulse width, 200 mJ pulse energy, and 40 Hz pulse rate to produce a range of seven total energy levels (ELs) per 100 cm^2, 750 J (EL1), 1500 J (EL2), 2250 J (EL3), 3000 J (EL 4), 3750 J (EL 5), 4500

J (EL 6) and 5250 J (EL 7) (Fig. 1). During the procedure, laser energy was conducted to the adipose tissue by a 600 μm optical fiber delivered through a 1 mm cannula. The cannula was inserted through an incision in the skin and was kept moving approximately 1 cm below the surface of the skin. The cannula was moved back and forth with an even, extracting motion at a rate of 2 cm/s.

To examine the immediate and long-term wound healing process following *in vivo* laser lipolysis, biopsies were performed immediately postoperatively and at 1 week, 2 weeks, and 3 months after laser exposure. The laser exposed sites were excised en bloc approximately with a 15 mm incisional biopsy, and the area was sutured immediately after. Tissue samples were fixed in 4% formalin immediately after biopsy and were stained with hematoxlyn-eosin for histologic analysis.

Results

Analysis of the immediate postoperative biopsy specimens revealed direct evidence of fat cell lysis. Compared to the uninjured polygonal fat cells of the control tissue before laser treatment (Fig. 2A), immediate biopsy following a total energy level of EL2 at 1444 nm showed histological characteristics of ruptured fat cell membranes and intact capillaries within the irradiated site (Fig. 2B). In the biopsy 2 weeks after the operation, closely stacked flattened adipocytes were noticeable (Fig. 2C). Histologically, features of regeneration, such as small-sized adipocytes with proliferating capillaries, were obvious in the biopsy at the 3-month follow-up (Fig. 2D and inset).

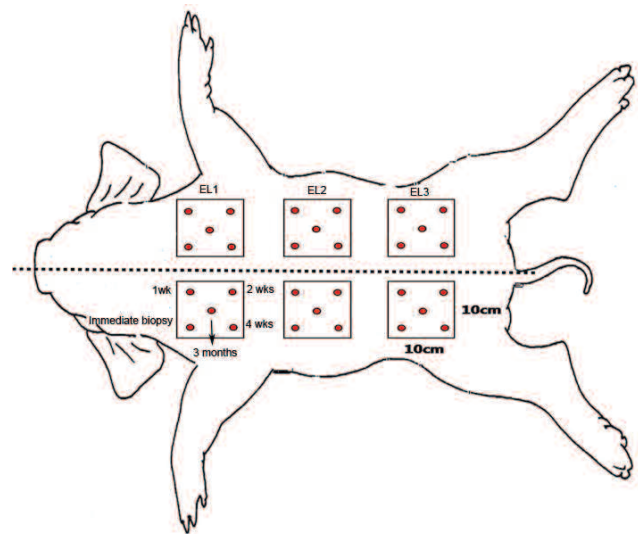An inflammatory reaction in response to lipolysis

Fig. 1. Schematic diagram of the study outline showing each corner of 10x10 cm sized six test sites with two sites for each energy level on the back of the first Micro-pig.

was histologically evident as early as 1 week postoperatively. The 1-week postoperative biopsy specimen treated with the 1444 nm laser at EL 2 showed mild inflammatory cell infiltration of mononuclear cells, including lymphocytes and histiocytes around the vessels (Fig. 3A). In contrast, a mild lobular inflammation was observed in the 1 week biopsy specimen treated with the 1444 nm laser at EL 3 (Fig. 3B). The degree of inflammation was higher with increasing energy levels. More intense lobular

inflammation associated with microcapillary hemorrhage was observed in the 1-week biopsy after laser treatment at EL7 (Fig. 3C).

The tissue damage resulting from the lobular inflammation was worsened as did capillary hemorrhage in the 2-week biopsy specimen at all energy levels. Compared to the mild fat necrosis in the 2-week biopsy from tissues treated with EL2 (Fig. 4A), treatment with EL3 showed more severe fat necrosis (Fig. 4B), including radially arranged crystalline spaces composed
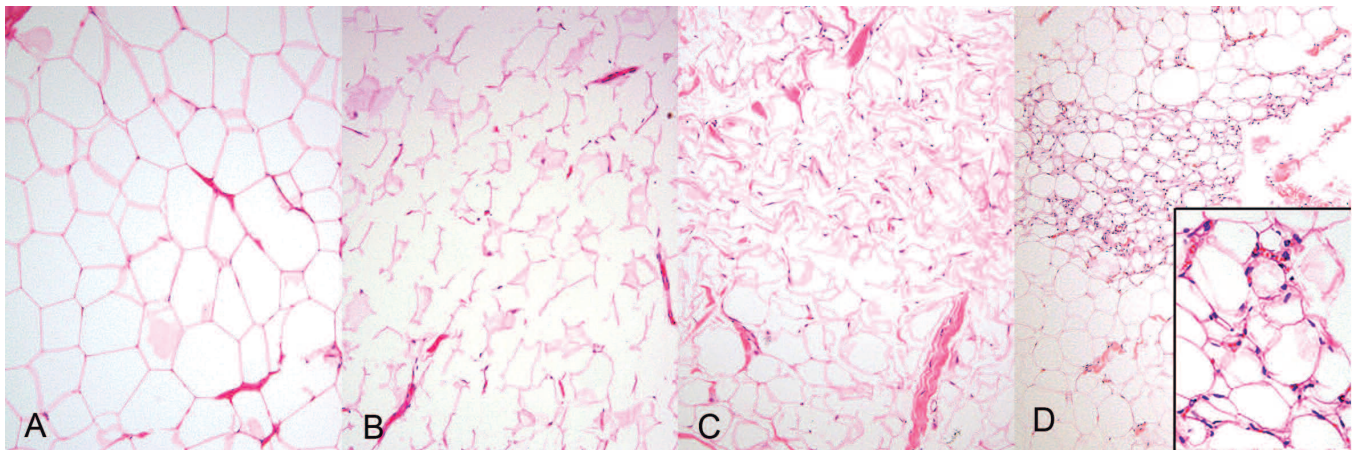

Fig. 2. Time course histologic evaluation of 1444 nm Nd:YAG laser lipolysis at a total EL 2. **A.** Uninjured polygonal fat cells of control tissue. **B.** Fat lysis of the immediate biopsy showing ruptured fat cell membranes and intact capillaries within the irradiated site. **C.** Closely stacked flattened adipocytes of the 2 week biopsy. **D.** Regenerated fat tissue with proliferating capillaries on the biopsy at the 3-month follow-up (inset). Hematoxylin-Eosin. x 400.
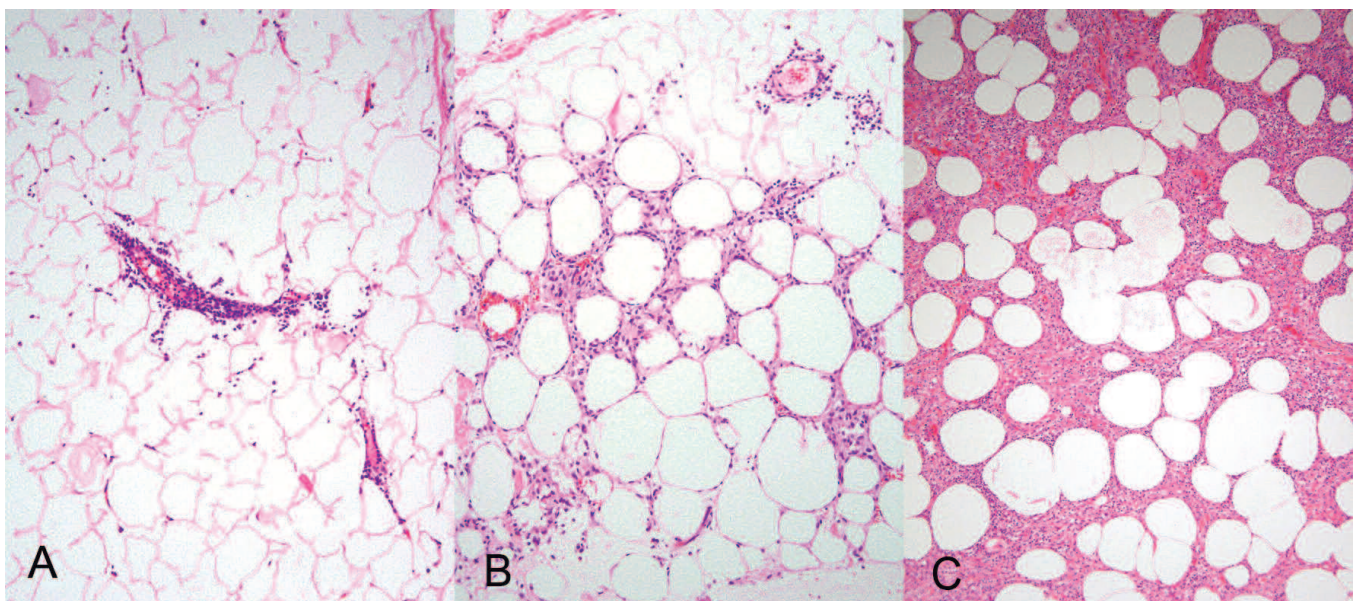

Fig. 3. Inflammation increases with increasing energy level. One-week postoperative biopsy specimen treated with the 1444 nm laser at EL 2 **(A)**, EL 3 **(B)**, and EL7 **(C)**. Hematoxylin-Eosin. x 400.

of triglycerides (Fig. 4C) and a few cholesterol cleft giant cells. Cholesterol cleft giant cells (Fig. 4D), generally reflecting vascular damage, were seen more frequently in the acute injuries associated with higher energy laser lipolysis at EL4 through EL7.

The energy-dependent extent of vascular damage was observed in the injured fat tissue treated with increasing laser energy levels. Compared to only hyalinization of the blood vessels at EL2 (Fig. 5A), capillary hemorrhage in the injured adipocytes was focally identified at EL3 (Fig. 5B) and more extensive capillary hemorrhage was noticeable at EL7 (Fig. 5C). In addition, microthrombi formation in the small vessels was observed as early as 1 week following treatment with EL4 (Fig. 5D).

While inflammation resolved over time in tissues treated at low energy levels, marked inflammation in the early-phase biopsies from tissues receiving higher energy levels resulted in scarring and cystic fat necrosis with persistent cholesterol granulomas in the later-phase biopsies. Whereas lobular inflammation was almost completely resolved and only a few lipid giant cells remained in the 4-week biopsy of EL2 and EL3 (Fig. 6A), obvious scarring, as well as cystic fat necrosis and many lipid giant cells were noticeable in the 4-week biopsy from EL4 and higher energy levels (Fig. 6B). In addition, lobular inflammation was still observed in the 4-week biopsy from the higher energy levels (Fig. 6C).
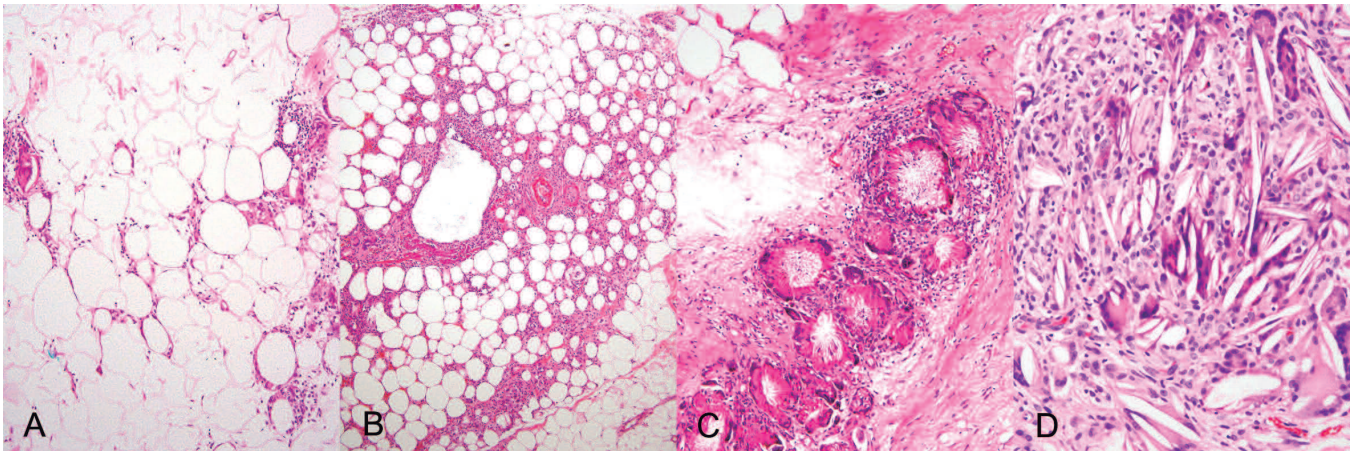

Fig. 4. The tissue damage worsened with increasing energy level as did vascular damage. Two-week biopsy specimen treated with EL2 **(A)** and EL3 **(B)** including radially arranged crystalline spaces composed of triglycerides **(C)** and cholesterol cleft giant cells **(D)**. Hematoxylin-Eosin. A, B, x 400; C, D, zoomed crop of x 400.
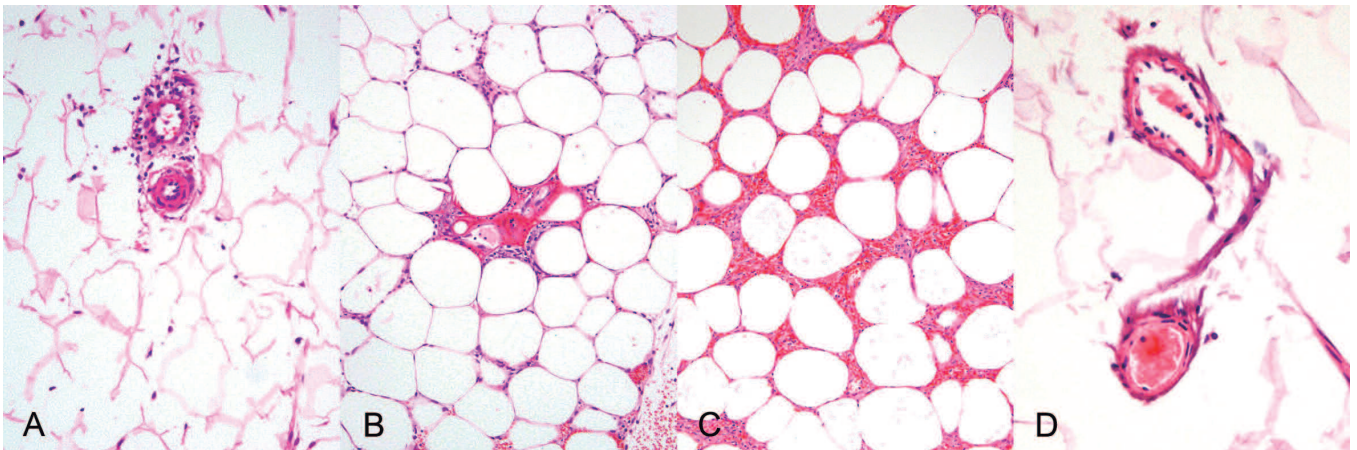

Fig. 5. Vascular damage increases with increasing energy level. **A.** Minimal capillary hemorrhage at EL2. **B.** Mild capillary hemorrhage of the injured adipocytes at EL3. **C.** Extensive capillary hemorrhage at EL7. **D.** Microthrombi in the small vessels as early as at 1 week biopsy with EL4. Hematoxylin-Eosin. Zoomed crop of x 400.

In the 3-month follow-up biopsy, regenerative features of the fat cells were characteristic with EL 2 treatment (Fig. 7A) and cystic fat necrosis with little scarring was a feature of EL3 treatment (Fig. 7B). Treatment with EL 4 led to mild scarring associated with cystic fat necrosis and persistent cholesterol giant cells (Fig. 7C). Treatment with EL 6 and EL7 resulted in increased fibrotic scarring with cystic fat necrosis and persistent cholesterol giant cells (Fig. 7D,E).

Adipocytes showing radially arranged crystalline spaces composed of triglycerides (Fig. 4C), frequently seen in fat necrosis, were usually resolved over time, but cholesterol granulomas (Fig. 4D) still remained in the later biopsy specimens from tissues treated with relatively high energy levels (from EL 4 to EL7). More serious demyelinating nerve injury was observed at the higher energy levels (Fig. 8A,B). The degree of coagulation of fat septal collagen and large vessels varied depending on the energy level. There was no histologically visible damage to the epidermis or dermis.

Discussion

Since the introduction of laser lipolysis, there have been many histologic studies on the effect of laser irradiation on fat tissue (Badin et al., 2005; Ichikawa et
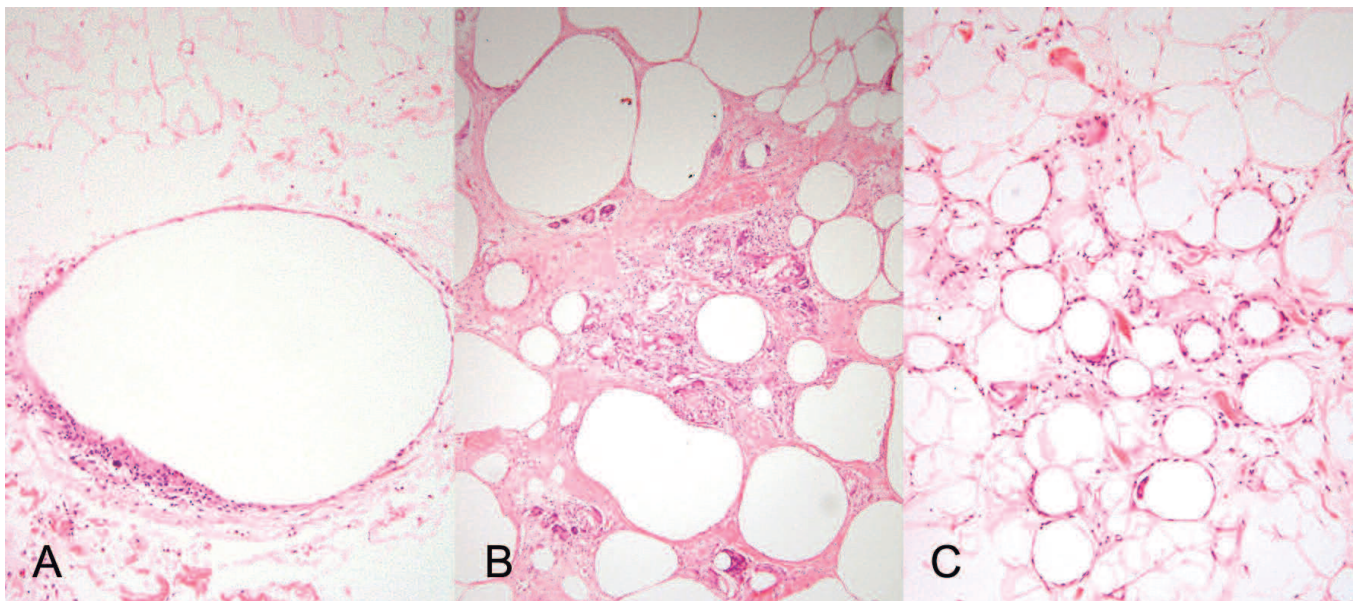

Fig. 6. Inflammation resolved over time and resulted in scarring and cystic fat necrosis depending on energy level. **A.** Resolved fat lysis in the 4-week biopsy with no fibrosis at EL3. **B.** Cystic fat necrosis and fibrosis in the 4-week biopsy at EL5. **C.** Presence of persistent lobular fat inflammation in the 4-week biopsy at EL5. Hematoxylin-Eosin. Zoomed crop of x 400.
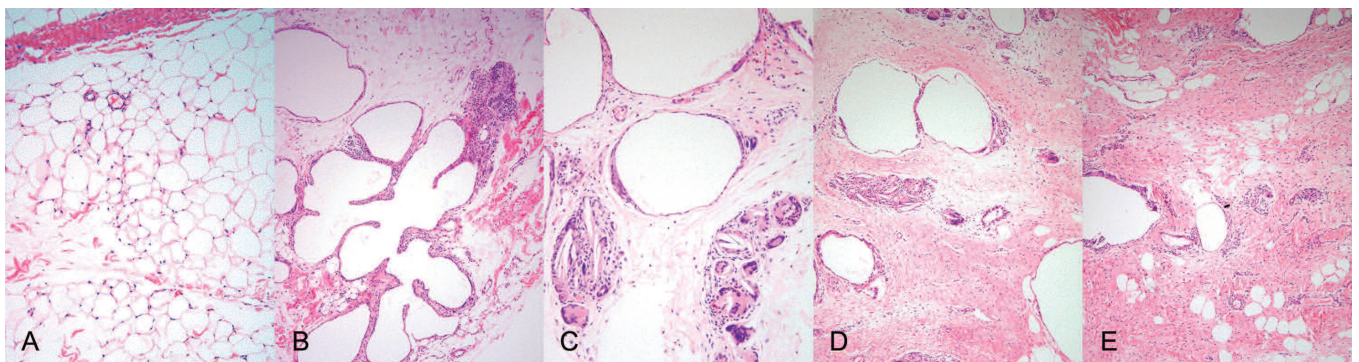

Fig. 7. Histological comparison of EL2(**A**), EL3(**B**), EL4(**C**), EL6(**D**), and EL7(**E**) at the 3-month postirradiation biopsy. Irradiation of ELs over EL4 resulted in serious fibrosis compared to the ELs 1 to 3 showing subsided inflammation without scarring. Hematoxylin-Eosin. x 400.

al., 2005; Goldman, 2006; Kim and Geronemus, 2006; Mordon et al., 2007; Khoury et al., 2008; O'Dey et al., 2008). However, all of these studies have focused on the immediate postoperative period. A recent long-term follow-up study discussed fat lysis after cryoinjury, but laser treatment and cryoinjury affect fat tissue differently (Tark et al., 2009). There have been no long-term histologic studies focusing on interstitial laser lipolysis.

Although the direct correlation of treatment effect by laser between the animal model and human model cannot be made, Tark et al. (2009) reported the 1444 nm Nd:YAG laser showed a greater lipolytic effect compared to the 1064 nm Nd:YAG laser in in vitro human fat experiments as well as in vivo mini-pig. In this long-term study, we conducted comprehensive histologic examinations of interstitial laser lipolysis using the 1444 nm wavelength Nd:YAG laser in a pig model to emphasize that the treatment dose is important in clinical application of a laser to human fat tissue.

Ruptured cellular membrane of the fat cells with no capillary rupture of the adipocytes was a characteristic feature of the biopsy specimens taken immediately following laser treatment in the current study. These findings are consistent with previous studies (Kuwahara et al., 2003; Ichikawa et al., 2005). Ichikawa et al. (2005) reported the same feature in human adipocytes after using a pulsed Nd:YAG laser for laser lipolysis. They found that degenerated and ruptured cell membranes caused by laser exposure were evident in the hematoxylin and eosin-stained tissues that underwent laser lipolysis, and dispersed lipids were visualized by scanning electron microscopy (Ichikawa et al., 2005). It appears indisputable that fat was lysed by interstitial laser irradiation in this study, and the regenerated features of the lytic fat tissue were also obvious in the 3-month follow-up biopsy when no significant damage to the fat capillaries was found.

In a recent study, Manstein et al. (2008) defined selective cryolysis as the intentional selective destruction of adipose tissue while cooling and sparing the skin. They noted that the fat tissue injured by cryolysis shows mostly a lobular panniculitis without vascular injury or overlying skin damage, and there was no abrupt disruption of fat cells. The characteristic feature of selective cryolysis is a gradual infiltration of lipid-laden macrophages resulting from phagocytosis of the lipid from extravasated monocytes in the injured fat tissue. This phagocytic process is a fat-removal mechanism associated with selective cryolysis. On the contrary, in the present study the abrupt rupture of fat cell membranes was a key feature of interstitial laser lipolysis, and inflammation appeared in response to lytic fat cells over time following treatment.

The fat cells store lipids primarily in the form of triglycerides. It has been reported that triglycerides are released by ultrasound which, like laser treatment, also works via thermal reaction (Grippaudo et al., 2000). When lipid flows out of the ruptured fat cells it is evacuated via the lymphatic system (Grippaudo et al., 2000). Excessive lipid beyond lymphatic drainage results in radially arranged crystalline spaces composed of triglyceride or cystic fat necrosis depending on the amount and extent of released lipid. While these forms of lipid are known to be eventually absorbed, cholesterol cleft giant cells can remain problematic by triggering sequential inflammation (Brodkey et al., 1996; Kiryu et al., 2000).

Cholesterol cleft giant cells are representative of prior vascular injury and develop at sites of hemorrhage and/or tissue necrosis secondary to an inflammatory
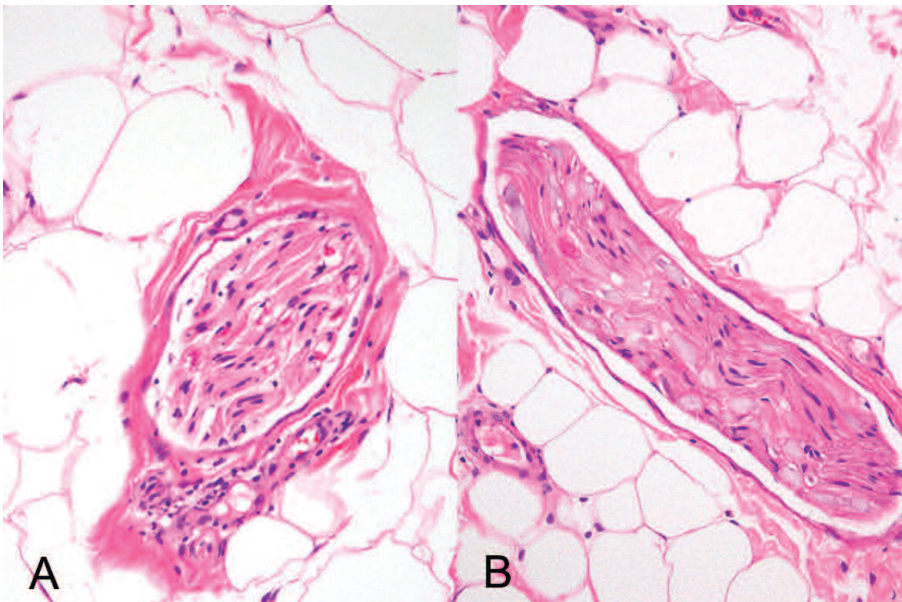

Fig. 8. Serious demyelinating nerve injury at the higher energy levels. Two-week biopsy at EL 4 **(A)** and EL 7 **(B)**. Hematoxylin-Eosin. Zoomed crop of x 400.

focus (Smith et al., 1977; Brodkey et al., 1996; Donelan et al., 2009). Histologically, they are characterized by the presence of empty needle-shaped crystals caused by the dissolution of cholesterol crystals in sections of tissue embedded in paraffin (Smith et al., 1977; Donelan et al., 2009). These crystals incite foreign body giant cell infiltration and repeated hemorrhaging, ultimately leading to cholesterol granuloma, an inflammatory reaction (Smith et al., 1977; Brodkey et al., 1996; Donelan et al., 2009). In the present study, cellular membrane components of erythrocytes destroyed during bleeding and ruptured fat cells were a source of cholesterol that formed cholesterol crystals, and the amount of persistent cholesterol cleft giant cells in long term follow-up was correlated with the laser energy level. Therefore, it could represent a worrisome marker that can help predict the eventual outcomes of laser lipolysis.

From our long-term follow-up study, we found that the histology of the injured fat tissue varied with different laser energy levels and responses of the fat cells, septal collagen, vessels, and nerves at the laser sites. The fat tissue underwent severe post-treatment inflammation and irreversible scarring with higher radiant fluxes (EL4 – EL7). At the lower radiant fluence levels (EL1 – EL3), on the other hand, the 1444 nm laser had an efficient lipolytic effect with less inflammation, leading to progressive thinning of the fat layer during the 3-month follow-up with no visible scar formation. To achieve an increased lipolytic effect it is necessary to have more inflammation caused by adipocyte apoptosis. Microvascular damage is also inevitable in lipolysis, and its degree appeared to determine the end results of lipolysis related to impaired wound healing. The 1444 nm wavelength has a very low absorption coefficient in hemoglobin compared to 1064 nm. This could contribute to low vascular damage from 1444 nm interstitial laser treatment (Roggan et al., 1999; Anderson et al., 2006). The 1444 nm wavelength seemed to offer advantages in that, at lower ELs, vascular injury and the resulting cholesterol granulomas, which are apparently related to increased inflammation and fibrosis, were not so evident in our histological assessments. Therefore, this study suggested that controlling the energy level is important for the eventual outcome of lipolysis.

Additionally, DiBernardo et al. (2009) also reported that impacts of over use of laser energy exist in human tissue. They observed the details of the tissue heating using 1064nm and 1320 nm wavelengths in the human adipose tissue and skin in conjunction with abdominoplasty, and proposed the optimal temperature range of each wavelength, due to thermal injuries such as skin necrosis and blistering caused by a significant temperature increase associated with high laser doses. Although they used the other wavelengths and mainly focused on the tissue heating of the skin rather than on the tissue response of the adipose tissue, their findings concurred with ours that the energy level is important for the eventual clinical outcome for both safe lipolysis and skin texture.

The removal or suctioning of the disrupted fat tissue immediately after laser lipolysis remains to be discussed to prevent undesirable tissue injury. Dudelzak et al. (2009) performed laser lipolysis using the 1064 nm Nd:YAG, and demonstrated no difference in clinical outcome whether or not post-laser suction was undertaken after laser lipolysis. While their intention was to show that laser lipolysis can be an effective treatment even without post-laser suction aspiration, the result could imply that post-laser suction to prevent the undesirable tissue contents may not be necessary, and controlling the energy level is more important for the eventual outcome of lipolysis. However, we propose that further study is needed to evaluate the effect of suction after laser lipolysis from the point of view of the energy level dependent tissue response including inflammation and scarring.

In summary, laser lipolysis is known to be a relatively safe treatment with fewer associated risks and side effects than surgical liposuction for clinical use. However, there is a risk of developing large fibrotic areas in the subcutis that lead to irregularity of the overlying skin texture, formation of palpable or visible nodules in the skin, and other possible sequelae. Our study showed that 1444 nm Nd:YAG laser-assisted lipolysis effectively destroys adipose tissue. However, significant treatment-associated complications, including irreversible scarring, could remain problematic during long-term follow-up in direct correlation with the amount of energy delivered. Therefore, appropriate parameters of laser lipolysis still need to be investigated to determine the precise safety guidelines for clinical application.

References

Anderson R.R., Farinelli W., Laubach H., Manstein D., Yaroslavsky A.N., Gubeli J. III., Jordan K., Neil G.R., Shinn M., Chandler W., Williams G.P., Benson S.V., Douglas D.R. and Dylla H.F. (2006). Selective photothermolysis of lipid-rich tissues: A free electron laser study. Lasers. Surg. Med. 38, 913-919.

Badin A.Z., Moraes L.M., Gondek L., Chiaratti M.G. and Canta L. (2002). Laser lipolysis: flaccidity under control. Aesthetic. Plast. Surg. 26, 335-339.

Badin A.Z., Gondek L.B., Garcia M.J., Valle L.C., Flizikowski F.B. and de Noronha L. (2005). Analysis of laser lipolysis effects on human tissue samples obtained from liposuction. Aesthetic. Plast. Surg. 29, 281-286.

Brodkey J.A., Robertson J.H., Shea J.J. 3rd. and Gardner G. (1996). Cholesterol granulomas of the petrous apex: combined neurosurgical and otological management. J. Neurosurg. 85, 625-633.

DiBernardo B.E., Reyes J. and Chen B. (2009). Evaluation of tissue thermal effects from 1064/1320-nm laser-assisted lipolysis and its

clinical implications. J. Cosmet. Laser. Ther. 11, 62-69.

Donelan K.J., Randall B.B. and Newby P.E. (2009). A 51-year-old man with sudden unexpected death. Brain Pathol. 19, 151-152.

Dudelzak J., Hussain M. and Goldberg D.J (2009). Laser lipolysis of the arm, with and without suction aspiration: clinical and histologic changes. J. Cosmet. Laser. Ther. 11, 70-73.

Goldman A. (2006). Submental Nd:Yag laser-assisted liposuction. Lasers. Surg. Med. 38, 181-184.

Grippaudo F.R., Matarese R.M., Macone A., Mazzocchi M. and Scuderi N. (2000). Effects of traditional and ultrasonic liposuction on adipose tissue: a biochemical approach. Plast. Reconstr. Surg. 106, 197-199.

Ichikawa K., Miyasaka M., Tanaka R., Tanino R., Mizukami K. and Wakaki M. (2005). Histologic evaluation of the pulsed Nd:YAG laser for laser lipolysis. Lasers Surg. Med. 36, 43-46.

Khoury J.G., Saluja R., Keel D., Detwiler S. and Goldman M.P. (2008). Histologic evaluation of interstitial lipolysis comparing a 1064, 1320 and 2100 nm laser in an ex vivo model. Lasers Surg. Med. 40, 402-406.

Kim K.H. and Geronemus R.G. (2006). Laser lipolysis using a novel 1,064 nm Nd:YAG laser. Dermatol. Surg. 32, 241-248.

Kiryu H., Rikihisa W. and Furue M. (2000). Encapsulated fat necrosis--a clinicopathological study of 8 cases and a literature review. J. Cutan. Pathol. 27, 19-23.

Kuwahara K., Gladstone H.B., Gupta V., Kireev V., Neel V. and Moy R.L. (2003). Rupture of fat cells using laser-generated ultra short stress waves. Lasers Surg. Med. 32, 279-285.

Manstein D., Laubach H., Watanabe K., Farinelli W., Zurakowski D. and Anderson R.R. (2008). Selective cryolysis: a novel method of non-invasive fat removal. Lasers Surg. Med. 40, 595-604.

Mordon S., Eymard-Maurin A.F., Wassmer B. and Ringot J. (2007). Histologic evaluation of laser lipolysis: pulsed 1064 nm Nd:YAG laser versus CW 980-nm diode laser. Aesthetic Surg. J. 27, 263-268.

O'Dey D., Prescher A., Poprawe R., Gaus S., Stanzel S. and Pallua N. (2008). Ablative targeting of fatty-tissue using a high-powered diode laser. Lasers Surg. Med. 40, 100-105.

Roggan A., Friebel M., Dorschel K., Hahn A. and Muller G. (1999). Optical properties of circulating human blood in the wavelength range 400–2500 nm. J. Biomed. Opt. 4, 36-46

Smith C., Thomas R. and Wright I. (1977). Temporal bone pathology in an active case of glue ear. Clin. Otolaryngol. Allied Sci. 2, 53-63.

Tark K.C., Jung J.E. and Song S.Y. (2009). Superior lipolytic effect of the 1,444 nm Nd:YAG Laser: Comparison with the 1,064 nm Nd:YAG laser. Lasers Surg. Med. 41, 721-727.