

# Ultrastructural assessment of cellulite morphology: clues to a therapeutic strategy?

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**Introduction and Aims:** Cellulite is a problematic condition affecting mostly women, characterized by a bumpy or nodular skin surface. Recent approaches with laser treatment have offered some promise. The present study sought to identify possible targets for laser treatment or light therapy through an ultrastructural investigation of the condition.

**Subjects and Methods:** Study subjects comprised 7 healthy Japanese female volunteers (Age range 37-46 yr, average 38.4) with cellulite, graded on the 4-point Nurnberger-Muller cellulite severity scale. Four patients were at grade 2 and 3 at grade 3. Three millimeter punch biopsies were obtained and routinely processed for light and transmission electron microscopy.

**Results:** Microphotography of specimens from cellulite patients demonstrated the presence of fibrotic septa which divided up larger clusters of adipose tissue into smaller packets, with the septa acting as a tethering system, thus producing the typical dimpling pattern. Ultrastructural findings showed proliferation of collagen and elastic fibers down into the cellulite tissue with compression of capillaries and congestion of arterioles, resulting in poor blood flow.

**Conclusions:** The histological and ultrastructural findings of cellulite clearly distinguish the condition from simple fat deposition. The remodeling of the fat layer into lobulated packets of lipocytes sequestered by fibrotic septa with a high proportion of elastic fibers would suggest the use of a fiber-based interstitial laser-assisted lipolysis system at an appropriate wavelength which might offer benefits through disruption of the septae through a photomechanical effect and lipolysis of the sequestered lipocytes. This could be followed by a course of near-infrared phototherapy to accelerate clearance of freed lipid and debris and reestablish the vascular system.

## Introduction

Cellulite <sup>1-3)</sup> is a condition in which localized metabolic failure in the fatty layer affects the body skin surface, and occurs mainly in women. The condition usually manifests itself as a bumpy or nodular surface contour of the skin in such areas as the waist, thighs, and abdomen in women. There are as yet few or no published studies which have definitively explained the disease state of cellulite, and it still remains uncertain why this condition occurs more frequently among women in the prime of their life and as to the localiza-

tion of affected sites. Cellulite should be considered for treatment not merely from the cosmetic viewpoint but also on account of its causal links with metabolic failure, circulatory failure and, occasionally, associated pain; however, treatment with topical and/or oral medications brings very little clinical benefit and it is recognized that even such alternatives as laser irradiation, ultrasound, and in some cases radiofrequency energy seem likely to fail in providing appreciable therapeutic effects. Simple circumferential reduction using low level red light therapy (LLLT) has attracted attention, <sup>4-6)</sup> but the morphology of cellulite differs from that of normal fat accumulation. More recently, laser diode-based low level light therapy at 532 nm had good effects on cellulite, but long-term efficacy remains to be demonstrated. <sup>7)</sup> Of potentially greater interest has

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been the emergence of interstitially optical fiber-delivered applications using a new wavelength based on a micropulsed Nd:YAG at 1444 nm for body and facial contouring.<sup>8-10</sup> We therefore conducted an ultrastructural study on cellulite with a pathophysiological interpretation of the condition, from which the existing and potential therapeutic approaches using laser or light therapy could be based.

**Subjects and Methods**

Seven healthy adult Japanese volunteers (aged 37 to 46 years, mean 38.4, all females of Fitzpatrick skin type III) were recruited for this study. Subjects with chronic illness, atopic dermatitis, contact dermatitis, photosensitivity, a history of scarring or poor wound healing, vascular disease or cutaneous disease were excluded. Written informed consent was obtained from each volunteer and the study was approved by the Ethics Committee of the Queen’s Square Medical Center.

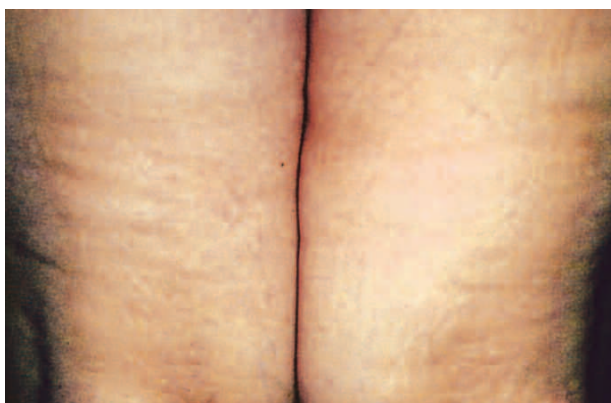
Cellulite is clinically graded using the 4 point

Numberger-Muller cellulite severity scale as illustrated in **Table 1**. The findings as seen in **Figure 1**, for example, would be categorized as Grade 2 because of the presence of modest dimples on the skin surface. Of the 7 patients in the present series, the condition was graded as Grade 3 in 4 patients and as Grade 4 in 3 patients.

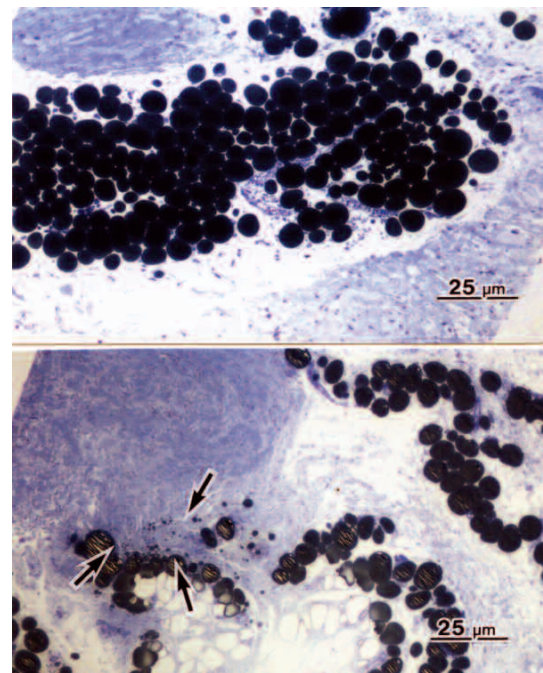
Three millimeter punch biopsies were obtained from the posterior thigh. Each specimen was fixed in glutaraldehyde (2.5%) and later in osmium tetroxide (1%). After dehydration through a graded ethanol series, the specimens were embedded in Epon 812 (Oken Shoji Co., Ltd, Tokyo, Japan), stained with toluidine blue, and examined by light microscopy. Ultrathin sections were obtained with an Ultracut N ultramicrotome (Reichert-Nissei, Tokyo, Japan) and a diamond knife. Sections were stained with oolong tea extract (OTE) for connective tissue,<sup>11</sup> uranyl acetate and lead

**Table 1:** The Nurnberger-Muller cellulite severity scale

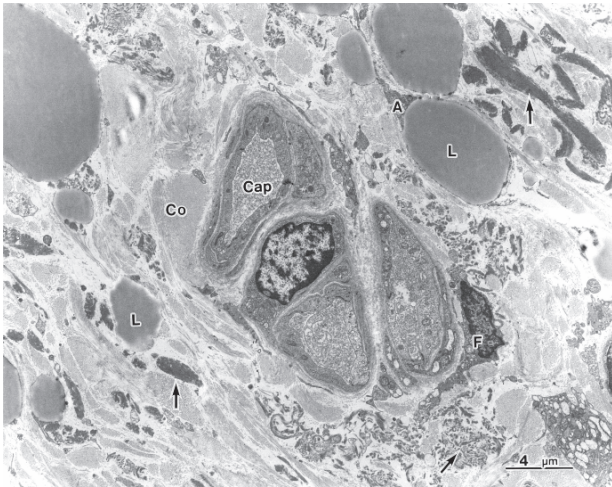
Stage	Standing	Supine	Pinch test
0	—	—	—
1	—	—	Dimpling present
2	Dimpling present	—	Dimpling present
3	Dimpling present	Dimpling present	Dimpling present



**Figure 1:** A view of Grade 2 cellulite on the Nurnberger-Muller scale. The cellulite in this patients was diagnosed as Grade 3 on account of the modest bumpiness of the skin surface visible on the pinch test and while standing. The skin surface presents the typical ‘orange peel appearance’ characteristic of cellulite.



**Figure 2:** Histology of normal fat layer (upper panel) and cellulite skin region (lower panel) In the upper photomicrograph, the adipose tissue presents as large clusters with surrounding coarse connective tissue. The lower photomicrograph of a skin section from a cellulite region shows that the large clusters of adipose tissue are sequestered by septa of connective tissue to form small clusters. Those clusters are closely linked to connective tissue in the periphery of the adipose tissue, where a number of lipid droplets that have proliferated are noted (arrow). (Toluidine blue stain, light microscopy, scale bars as shown)



**Figure 3:** Cellulite skin region. This photomicrograph of a skin region shows proliferation of small lipid droplets (L) which can be seen synthesized in an adipocyte (A), and hemostasis in the capillary lumen (Cap) are noted. Proliferation of collagen fibers (Co) and elastic fibers (arrow) are also evident in this region. (Transmission electron microscopy with double staining using oolong tea extract for connective tissue: Scale bar as shown)

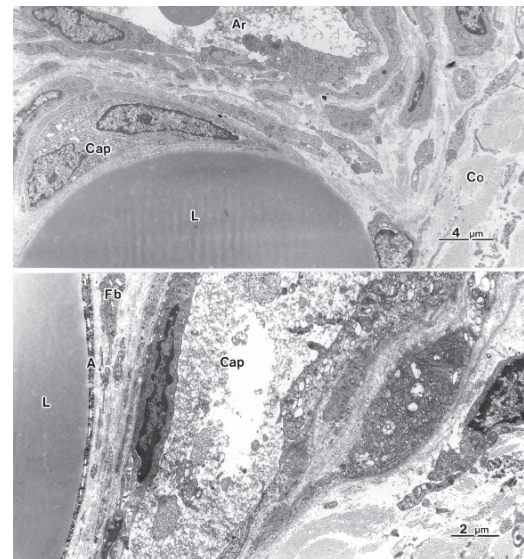
citrate prior to electron microscopic examination (75 kV, Hitachi H-7500, Hitachi, Tokyo, Japan).

## Results

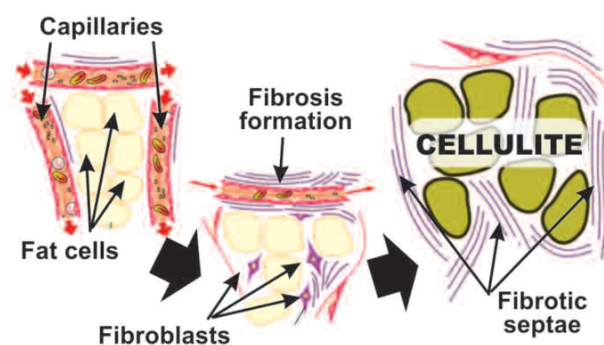
As seen in the optical photomicrograph (toluidine blue stain) of a normal control (**Figure 2**), the adipose tissue presented as large clusters with surrounding coarse connective tissue. The lower panel of **Figure 2** shows a skin section from a cellulite region demonstrating that large clusters of adipose tissue were sequestered by septa of connective tissue to form small clusters. Those clusters were closely linked to connective tissue in the periphery of adipose tissue, where a proliferation of lipid droplets could be observed.

The ultrastructural study demonstrated lipid droplets synthesized in an adipocyte and hemostasis in the capillary lumen of a region with a proliferation of lipid droplets (**Figure 3**). Proliferation of collagen fibers and elastic fibers could also be noted in this region.

A photomicrograph of a region at the periphery of the adipose tissue (**Figure 4, upper**) shows compression of a capillary by fat cells and congestion in an arteriole. Collagen fibers and fibroblasts could be found on the surface of an adipocyte enclosing a large lipid droplet. (**Figure 4, lower**). Capillary endothelial



**Figure 4:** Cellulite skin region. A capillary (Cap) with narrowing of the lumen due to fat cell compression and congestion of an arteriole (Ar) are noted in the periphery of the adipose tissue. (**Upper panel**): L: lipid droplet, Co: collagen fibril. Collagen fibers and fibroblasts (FB) are found on the surface of an adipocyte (A) possessing a large lipid droplet (L). (**Lower panel**): Capillary (Cap) endothelial cells presenting a practically normal picture and those having vacuolar degeneration are noted. (Transmission electron microscopy with double staining using oolong tea extract for connective tissue: Scale bar as shown)



**Figure 5:** Histogenesis of cellulite. As regards the pathophysiological genesis of cellulite, it is considered that cellulite represents a condition in which fibrosis arises with the proliferation of fibroblasts around adipose cells (center) in association with progressing peripheral circulatory failure and metabolic failure in normal tissue (left), thereby leading to progressively enhancing metabolic failure in adipose tissue (center) and eventually to adipose tissue degeneration and advanced fibrosis in the surrounding tissues (right).

cells presented a practically normal picture in addition to some with vacuolar degeneration.

## Discussion

Clinically, cellulite is recognized as presenting the so-called 'orange peel appearance.' This cutaneous change, first reported with the term 'non-inflammatory complex cellular dystrophy' in 1920<sup>12)</sup>, can also be historically confirmed through paintings created in the seventeenth century. Cellulite differs from what is called obesity. Obesity results from enlargement and an increase in the number of adipocytes but is not confined to any particular sites such as around the waist and thighs. Cellulite, on the other hand, has been described to be characterized by clinical changes of the skin and has a propensity to most frequently involve the dorsal aspect of the thigh, followed in order by around the abdomen and flank<sup>1)</sup>.

There are few or no published studies which have as yet precisely described the disease state of cellulite, and it remains unclear why this disorder occurs more frequently among women in the prime of their life and as to localization of affected sites<sup>2,3)</sup>. Besides the considerable importance of genetic factors, the following factors have been documented: predominance among females, relative uncommonness in the yellow races as compared to Caucasians, and an increased total lipid level due to hyperinsulinemia and fat production stemming from excessive carbohydrate intake restriction<sup>1,3)</sup>. The disorder is thought also to be related with hormonal imbalance because the disorder is noted frequently after pregnancy<sup>13)</sup>, as well as with aging-related changes, circulatory disorders, a poor posture, and excessive alcohol intake<sup>1,3)</sup>.

Physiological differences between adipocytes in cellulite and normal adipocytes are yet to be clarified. According to a study on autopsy cases reported by Pierard et al.<sup>14)</sup>, however, a substantial difference was observed to exist in connective tissue at the border between the dermis and the fat layer. These authors reported that, in cellulite, there were tortuous connective tissue components lying from the panniculus adiposus into the dermis, where an increase in  $\alpha$ -actin filaments abundant in myofibroblasts was found. Connective tissue changes in the striae of normal skin are in a horizontal direction, whereas in cellulite, those changes are in a vertical direction, which may account for the disorder<sup>15)</sup>. In the ultrastructural study we conducted, the mode of connective tissue coursing at sites of cellulite could not be ascertained. Proliferation of connective tissue, nevertheless, was demonstrable and

it could also be noted that large clusters of adipose tissue were sequestered by septa of connective tissue, dividing into small clusters. Such lobulation and sequestration of adipose tissue were confirmed by CT scanning<sup>16,17)</sup>. Normal adipose tissue has spaces to allow it to freely move on skin compression, whereas cellulite-affected adipose tissue is characterized by hardness of the fat layer *per se*.

Increased mitosis of adipose cells may be mentioned as a characteristic feature of cellulite tissue. The present study has demonstrated a noticeable increase of adipose cells possessing small lipid droplets in the periphery of adipose tissue, along with proliferation of connective tissue. The connective tissue showed proliferation not only of collagen fibers but also elastic fibers, accounting for its increased hardness in tissue texture. Hemostasis and congestion of the capillary lumen were noted. These seemed very likely to be due to adipose cell impingement on the vessels. Congestion was evident in the arteriolar lumina. Capillary endothelial cells occasionally showed vacuolar degeneration. Histologic findings of such vascular changes have been reported<sup>19,20)</sup>, and the present ultrastructural study has verified the presence of dysfunction, in the form of vacuolar degeneration, of capillary endothelial cells.

As regards the pathophysiological genesis of cellulite, it is considered that cellulite represents a condition in which fibrosis arises in adipose tissue in association with peripheral circulatory failure and metabolic failure, thereby leading to progressively enhancing metabolic failure in adipose tissue and eventually to adipose tissue degeneration and advanced fibrosis in the surrounding tissues (**Figure 5**).

Procedures such as simple massage with or without near-infrared irradiation have been applied in the treatment of cellulite, yet without any gratifying results, and other treatment modalities including radiofrequency/microwave energies have also been used<sup>20,21)</sup>.

On the other hand, recent reports have proven the efficacy of low level light therapy (LLLT) using red laser diodes to obtain circumferential improvement in patients requesting fat reduction,<sup>5,6)</sup> but these results could not easily be extrapolated to the treatment of cellulite due to the extensive morphological differences between normal fatty tissue and cellulite tissue, such as the presence of the septa and the relative firmness of the affected tissues. A small pilot study would, however, be interesting.

Laser diode-based LLLT with a visible green wavelength has, however, been reported as efficacious in the treatment of cellulite. In a controlled study with

a reasonably sized population.<sup>7)</sup> Subjects in the treatment group had a 1 – 2 grade improvement in their cellulite on the Nurnberger-Muller scale compared with none in the control group. The mechanisms, however, were not explored, and long-term follow-up results are awaited.

When the morphological aspects of cellulite are considered, a possible approach could involve the interstitial use of the 1444 nm line of the Nd:YAG laser, which has proved highly effective in body- and facial contouring.<sup>8-10)</sup> Absorption in both fat and water is extremely high at this wavelength associated with very effective lipolysis and thermal confinement for safety, and the micropulsed 100  $\mu$ s beam with very high peak powers at the tip of the 600  $\mu$ m fiber delivers a powerful photoacoustic effect, rupturing target tissues photo-mechanically. This combination might prove interesting to destroy the fibrous septa encapsulating the lipocytes while lysing the lipid in the fat cells. Dibernardo and colleagues used a similar wavelength (1440 nm) with a side-firing cannula to treat cellulite with some success, and a 6-month follow up.<sup>21)</sup>

Mild to moderate improvement in cellulite was reported in some, but not the majority, of the subjects by Truitt and colleagues in a transdermally-applied long-pulsed 1064 nm Nd:YAG with skin cooling<sup>6)</sup> 22). The authors applied both high and low fluence parameters, and interestingly the low fluences produced better results. However, the 1444 nm wavelength has

been shown to be superior to the 1064 nm Nd:YAG laser because of the duality of high absorption in fat and water of the former. A pilot study with the 1444 nm wavelength is eagerly anticipated to see if the theory would be borne out in practice.

An adjunctive approach might be to follow one of the above invasive procedures with LED-LLLT at 830 nm, which is known to penetrate well into the subdermal layer and has been shown to enhance wound healing, including improved remodeling, and accelerate macrophage activity.<sup>23)</sup> This might encourage the required scavenging of debris from the area and deliver enhanced remodeling of the affected subcutaneous tissue without reformation of the constricting fibrous septa.

## Conclusions

To treat cellulite with any degree of long-term efficacy obviously requires addressing the abnormal morphology of cellulite tissue followed by remodeling of the architecture of the septa and enclosed lipocytes, and some of the approaches outlined above have obviously gone some way to achieving this. Those which have been suggested may also have promise. In addition, the lifestyle of the patients must be examined to alleviate any signs of the metabolic syndrome, which is often associated with severe cases of cellulite.

## References

- 1: Rossi ABR, Vergnanini AL: Cellulite-a review. *JEA* 14:251-262, 2000
- 2: Almeida MC, Serrano CS, Roldan JR, Rejano JJ: Cellulite's aetiology: a review. *JEA* 27:273-278, 2013
- 3: Lotti T, Ghersetich I, Grappone C: Proteoglycans in so-called cellulite, *Br J Dermatol*, 29:272-274, 1990
- 4: Elm CM, Wallander ID, Endrizzi B, Zelickson B: Efficacy of a multiple diode laser system for body contouring. *Lasers Surg Med*, 2011; 43: 114-121.
- 5: Jackson RF, Stern FA, Neira R, Ortiz-Neira CL, Maloney J: Application of low-level laser therapy for noninvasive body contouring. *Lasers Surg Med*, 2012; 44: 211-217. Epub 2012 Feb 23.
- 6: McRae E, Boris J: Independent evaluation of low-level laser therapy at 635nm for non-invasive body contouring of the waist, hips, and thighs. *Lasers Surg Med*, 2013; 45: 1-7.
- 7: Jackson RF, Roche GC, Shanks SC: A double-blind, placebo-controlled randomized trial evaluating the ability of low-level laser therapy to improve the appearance of cellulite. *Lasers Surg Med*. 2013; 45: 8: Holcomb JD, Turk J, Baek SJ, Rousso DE: Laser-assisted facial contouring using a thermally confined 1444-nm Nd-YAG laser: a new paradigm for facial sculpting and rejuvenation. *Facial Plast Surg*, 2011; 27: 315-330. Epub 2011 Jul 26.
- 9: Lim SD, Youn JI, Kim WS, Kim IH, Hwang TS et al.: Comprehensive histologic analysis of interstitial lipolysis with the 1444 nm wavelength during a 3-month follow-up. *Histol Histopathol*. 2012; 26: 1375-1382.
- 10: Jung YC: Preliminary experience in facial and body contouring with 1444 nm micropulsed Nd:YAG laser-assisted lipolysis: a review of 24 cases. *Laser Therapy*, 2011. 20: 39-46.

- 11: Sato S, Sasaki Y, Adachi A, Dai W, Liu XL, Namimatsu S. Use of oolong tea extract (OTE) for elastin staining and enhancement in ultrathin specimens. *Med. Electron. Microsc*, 2003; 36: 179-182.
- 12: Scherwitz C, Braun-Falco O: So-called cellulite. *J Dermatol Surg Oncol*, 4: 230-234, 1978
- 13: Nurnberger F, Muller G. So-called cellulite: an invented disease. *J Dermatol Surg Oncol*, 4: 221-229, 1978
- 14: Pierard GE, Nizet JL, Pierard-Franchimont C. Cellulite: from standing fat herniation to hypodermal stretch marks. *Am J Dermatopathol*, 22: 34-37, 2000
- 15: Agache P, Ovide MT, Laurent R. Mechanical factors in striae distensae. In: Moretti GR, Reborja A, editors. *Striae distensae*. Milan: Symposium publication Division Brocades pp87-96, 1976
- 16: Mirrashed F, Sharp JC, Krause V: Pilot study of dermal and subcutaneous fat structures by MRI in individuals who differ in gender, BMI, and cellulite grading. *Skin Res Technol*, 10: 161-168, 2004
- 17: Merlen JF, Curri SB. Anatomico-pathological causes of cellulite. *J Mal Vasc*, 9(suppl A): 53-54, 1984
- 18: Curri SB, Merlen JF: Microvascular disorders of adipose tissue. *J Mal Vasc* 11: 303-309, 1986
- 19: Krueger N, Sadick NS: New-generation radiofrequency technology. *Cutis*, 91: 39-46, 2013
- 20: Gold MH: Cellulite - an overview of non-invasive therapy with energy-based systems. *J Dtsch Dermatol Ges*, 10: 553-8, 2012
- 21: Dibernardo B, Sasaki G, Katz BE, Hunstad JP, Petti C, Burns AJ: A Multicenter Study for a Single, Three-Step Laser Treatment for Cellulite Using a 1440-nm Nd:YAG Laser, a Novel Side-Firing Fiber, and a Temperature-Sensing Cannula. *Aesthet Surg J*. 2013 Mar 27. Epub ahead of print
- 22: Truitt A, Elkeeb L, Ortiz A, Saedi N, Echague A, Kelly KM: Evaluation of a long pulsed 1064-nm Nd:YAG laser for improvement in appearance of cellulite. *J Cosmet Laser Ther*, 2012; 14:139-144.
- 23: Kim WS and Calderhead RG: Is light-emitting diode phototherapy (LED-LLLT) really effective? *Laser Therapy*, 2011; 20: 205 – 215.