Fat Liquefaction:

Effect from Low Level

Laser Energy on Adipose Tissue.

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Background and Objective: **Low level laser (LLL) energy has been used increasingly in the treatment of a broad range of conditions, and has improved wound healing, reduced edema, and relieved pain of various etiologies. This study inquired whether the Erchonia 635 nm LLL affected in vivo adipose tissue and impacted the procedural implementation of Lipoplasty / Liposuction techniques.**

Study Design/Material and Methods**: The experiment investigated the effect of Erchonia 635 nm, 10 mW electric diode, with exclusive energy dispersing optics, at dosages of 2.4 J/cm² and 3.6 J/cm² on the human adipose tissue of ten (10) healthy women. The tissue samples were irradiated for 0- 2-4 and 6 minutes with and without tumescence solution, then studied using the protocol of Transmission**

Electron Microscopy (TEM) and Scanning Electron microscopy (SEM). An excess of 180 images were recorded and professionally evaluated.

***Results*: SEM and TEM results show the normal adipose tissue, without laser exposure, which appears as a grape-shape node. At 4 minutes laser exposure, 80% of the fat is released from the adipose cells, and at 6 minutes, laser exposure, 99% of the fat is released from the adipocyte cells. The released fat is collected in the interstitial space. Images of the adipose tissue taken at 60,000 magnification show a transitory pore and complete deflation of the adipocyte.**

***Conclusions*: The low level laser energy has an impact effect over the adipose cell consisting in opening a transitory pore, in the cell membrane which permits the fat content to go from inside to outside the cell. The cells interstice and capillaries are intact. The low-level laser assisted lipoplasty is an impacted procedural implementation of lipoplasty techniques.**

KEYWORDS: Lipoplasty, laser assisted surgery, adipose cell, SEM, TEM, laser-tissue interaction.

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INTRODUCTION

The science of Lipoplasty has advanced significantly since its 1921 inception when Charles Dujarrier (1) of France, attempted to remove subcutaneous fat from a dancer's calves using a uterine curette. Although Dujarrier's results were less than acceptable, he proved the potential to beautify the human body as a viable practice. Lipoplasty and it's beginning rudimentary tools were improved upon through the innovative thinking of professionals such as Babcock (2), who in (1939), initiated techniques to contour the breast and abdomen, Babcock was followed by Pitanguy (3) (1967), Regnaud (4) (1975), Illouz (5) (1976), Jackson (6) (1978), and Juri (7) (1979), all of whom contributed to the growing popularity of contour operations. The process was revolutionized in 1980 when Schrudde (8) introduced Lipexeresis as a means of eliminating local adiposity. Fournier and Otteni (1983) used uncut edge cannulas for contouring bodies through Lipolysis. Fodor (9) described the superwet technique in 1986 , Jeffrey Klein's (10,11) development of the tumescent technique (1987) which allowed near bloodless liposuction using only local anesthesia increased the popularity of Lipoplasty. In 1993, the internal ultrasound technique was developed by Michael Zocchi (12) and, in 1998, the external ultrasound was developed by Silberg (13), Neira et al (14) presented at the World Congress on Liposuction in Michigan, Oct 2000, a new liposuction technique demostrating liquefaction of fat during a liposuction procedure utilizing a low level laser device. All of these techniques have improved the surgical procedure in varying degrees of contribution; some have reduced risk to the patient, while others have expedited the process, yet all ultimately aim at decreasing fat panicles and thereby facilitating fat extraction. Each is a testimony of the developer's ability to use knowledge learned in a dynamic model in order to expand the science and application of Lipoplasty.

Combining low-level laser energy to the established practice of Lipoplasty, in order to create a new modality (15), is a result of the same dynamic application of knowledge. In understanding that, low level lasers (LLL) have been commonly used in the area of physical therapy and as anti-inflammatory devices (16). Low-level laser therapy (LLLT) is defined as a treatment with a dose rate that causes no immediate detectable temperature rise of the treated tissue and no macroscopically visible changes in tissue structure (16). Over the past decade, low-level laser energy has been used increasingly in the treatment of a broad range of conditions, and has improved wound healing, reduced edema, and relieved pain of various etiologies (16). Commonly used lasers are the Helium-Neon laser (632 nm wavelength) and Semiconductor-Diode Lasers (with a broad range of wavelengths between 600-800 nm). The dosage is a magnitude used to define the laser beam energy applied to the tissue. Normal units for the dosage are given in Joules/cm^2, and it is calculated as the laser power measured in mW, multiplied by treatment time in seconds and divided by area in cm^2 of the laser spot directed toward the tissue.

Many studies have been conducted on the most efficient use of and the most effective application of laser energy, (23) the results of which where dependent on three factors:

- Coherent light verses non-coherent light
- Power
- Wavelength

COHERENT LIGHT

Frohlich, a Doctor of Physics, specializing in Biological Coherence; predicted, on the basis of quantum physics, that the living matrix must produce coherent or laser-like oscillations to thrive in a state of health (1960). Coherent vibrations recognize no boundaries, at the surface of a molecule cell, or organism – they are collective or cooperative properties of the entire being. As such, they are likely to serve as signals that integrate processes such as growth, repair, defense and the functioning of the organism as a whole. Each molecule, cell, tissue and organ has an ideal resonance frequency that coordinates its activities. Light energy can directly influence the body's systematic defense and repair mechanisms by manipulating and balancing the vibratory circuits. Research on electrically polarized molecule arrays reveals that interactions repeated by the millions of molecules within a cell membrane give rise to huge coherent or laser like vibrations proving that crystalline components of the living matrix act as coherent molecular antennas, radiating and receiving signals.

OPTIMUM WAVELENGTH

Research supports 630 -- 640nm as the optimum (24, 25, and 26) wavelength because it facilitates biomodulation. Furthermore, this range of wavelength promotes proliferation of fibroblasts and keratinocytes, increases skin circulation, microcirculation and diminishes scar tissue. 630 -- 640nm was proven more effective by 6 –14 % minimum on wound healing when compared to 6 other lasers.

OPTIMUM POWER

From our researched-based knowledge, we know most lasers emit too high a power level to be effective for augmentation to Lipoplasty. Power density and exposure time results show that laser power below 2.91milliWatt could enhance cell proliferation whereas higher power had no effect. Stimulatory effects are most pronounced at irradiation times between 0.5 and 6 minutes. The biological law known as Arnat - Schultz states that weak stimuli excite physiological activity, moderately strong ones retard it, and very strong ones arrest it. Reiterating laboratory analysis, that 10milliWatt laser is more effective than a 100milliWatt laser for cell mitosis.

After assessing all known variables to develop our hypothesis, specifically, that the application of low level laser energy, effectively administered in accordance with established criteria addressing coherence, wavelength, and power; and applied to proven Lipoplasty / Liposuction processes, would in fact, result in a significantly safer, less time sensitive and relatively trauma free procedure. Identifying this procedure as Laser-Assisted Liposuction (LAL), our multi-discipline team of experts set out to establish proof for our hypothesis utilizing scientifically proven testing methodologies measured against industry-established metrics.

PROCESS / PROTOCOL

The impetus for this research was the plastic surgeon's, and author of this paper, repeated observation that fat was easily drained during a liposuction procedure when cold laser was applied to reduce inflammation and minimize pain during surgery. These observations were showing the same results for both pre and post-surgical period. When only the external laser beam was applied in a classical tumescent technique procedure, irradiation facilitated fat removal. After these factual findings and the cumulative clinical evidence, the clinical team studied the possible cellular effects in order to detect and achieve microscopic evidence regarding the suspected, possible effects of laser irradiation. Random samples were taken and submitted to VIS light microscopy. Although the initial results of the optical studies were non-conclusive due to initial sample testing procedures, the clinical team decided to continue the case study because the preliminary clinical evidence achieved by the plastic surgeon, co-author of this paper, was clearly impressive.

The clinical team decided to send samples to SEM and TEM studies. Both SEM and TEM were performed on superficial and deep fat samples to establish cellular effects correlated to the penetration depth of the laser beam after application of the tumescent technique. Samples without tumescent technique and exposure to laser for 0, 4, and 6 minutes, were also taken. Results indicated that the tumescent technique facilitates laser beam penetration and intensity; fat liquefaction is thus improved.

Fat samples were processed as follows:

1. The adipose tissue taken from the abdominoplasty without laser exposure was given to do SEM and TEM studies.

2. Application of tumescent technique and exposure to laser beam for 4 minutes.

3. Application of tumescent technique and exposure to laser beam for 6 minutes.

4. Without the use of tumescent solution, in vitro exposure of adipose tissue to laser beam for 4, and 6 minutes was performed and compared with samples without laser exposure (Zero minutes).

5. The adipose cell membrane was also studied in detail with TEM in order to clarify the suspected pore.

MATERIALS AND METHODS

Ten healthy women who had undergone corporal laser-assisted Lipoplasty (L.A.L.) were selected for random fat sampling. Patients' follow-up was 24 hours after surgery and up to 12 months after the procedure.

The tumescent technique was applied followed by external laser therapy using a low-level energy diode laser, with a nominal wavelength at 635 nm, and a maximal power of 10 mW. Cellular effects were studied in samples following 2 minutes, 4 minutes and 6 minute exposure times irradiating the tissue externally through the skin. Using the same 10mW power low level laser beam, a total amount of energy deposited on the tissue at 2 minutes was 1.2 joules, for 4 minutes was 2.4 joules and at 6 minutes was 3.6 joules.

Additionally, samples of laser treated tissue were taken from superficial and deep fat from the infraumbilical middle line in all patients studied. Biopsies were taken with a scalpel (no. 11) from tissue extracted from abdominoplasty and then introduced in a 0.1 cc glutaraldehyde phosphate 2.5% buffer at pH 7.2 and 4 °C. Furthermore, fat samples extracted without the tumescent technique were also studied. After this procedure, fat samples were irradiated following the aforementioned sequential laser exposure.

These samples were then submitted to scanning electron microscopy (SEM) and transmission electronic microscopy (TEM) to study the effect of the cold laser beam on the fat cell. The protocols used to study these samples are presented in the Appendix. Since no major differences were observed between time exposures of 2 and 4 minutes regarding changes in fat in adipose tissue, the samples were to be standardized to those taken for 4 and 6 minutes exposure time, in which cell effects could be observed under SEM and TEM.

RESULTS

Scanning (SEM) and transmission electronic microscopy (TEM) Findings:

1.Application of tumescent technique without exposure to the laser beam:

You can see the adipose without laser exposure, SEM shows a tri-dimensional picture of the adipocytes; contours are regular and the traditional grape-cluster shape appears (Figure 1). This tissue has received tumescent solution, but has not been exposed to the laser beam.

2.Application of tumescent technique and exposure to laser beam for 4 minutes. Partial disruption of the adipose cell membrane was observed; several cells without disruption of the cellular membrane were preserved. The adipose cells lost their round shape, and fat spread into the intercellular space going from inside to outside the cell (Figure 2A, 2B)

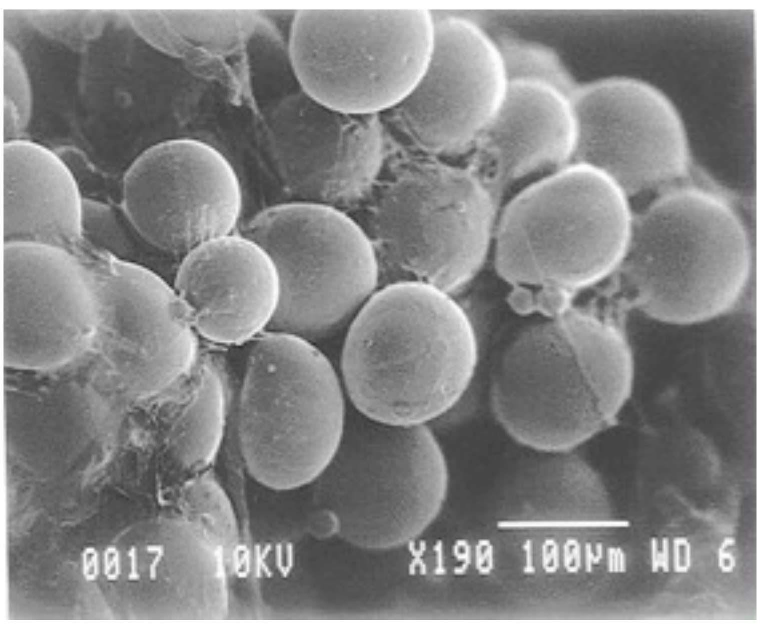

Figure 1 -- SEM Picture. The adipocytes have round shape; contours are regular having a grape-cluster shape. Picture magnification of 190x

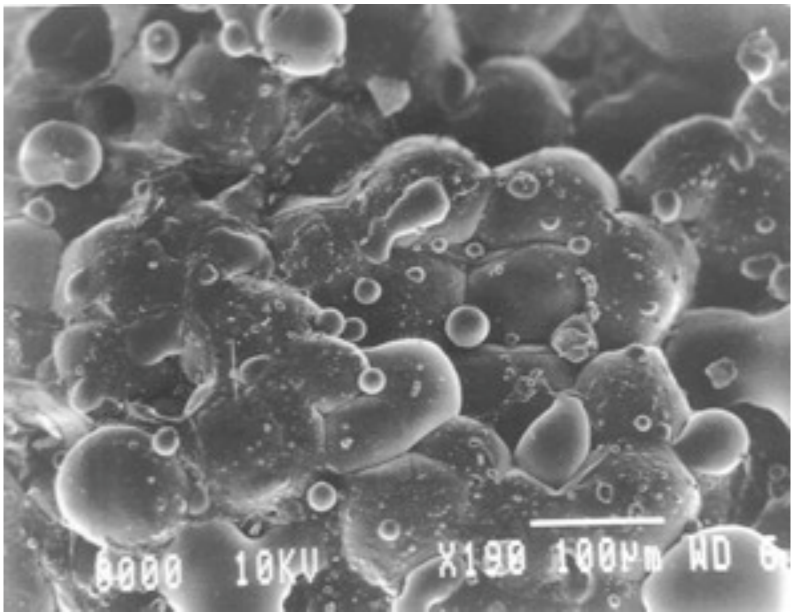

Figure 2A -- SEM picture. Application of the laser beam for 4 minutes Only few adipocytes are liquefied, there is preservation of some cell membrane, some has lost its original shape 190Xmagnifications times

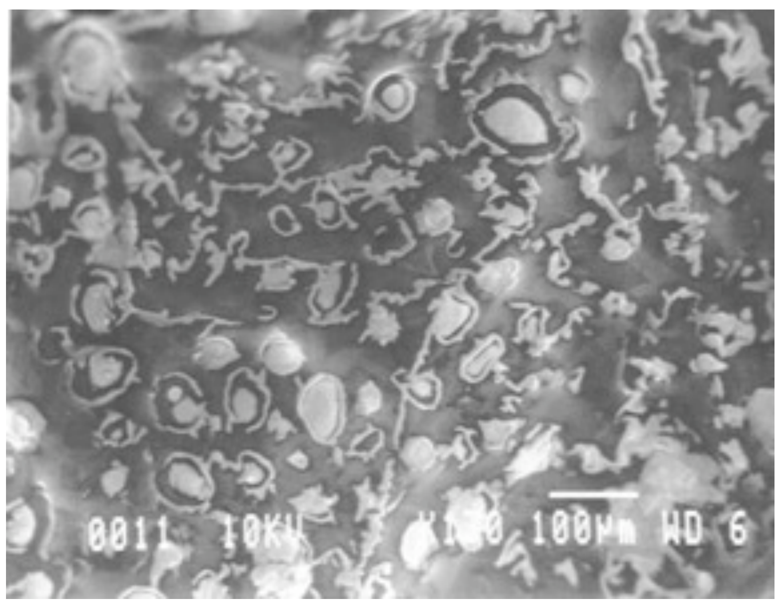

Figure 2B -- SEM picture. Adipocyte exposure to the laser beam for 4 minutes, adipocytes have lost its round shape some have a star and oval shape. 130Xmagnification times

3. *Application of tumescent technique and exposure to laser beam for 6 minutes.* Microscopic evidence was found proving that fat was completely removed from the cells, and retained in the interstitial space. Some disruption of the connective tissue was also observed; other structures, such as the capillaries and the remaining interstitial space, were however preserved (Figure 3.)

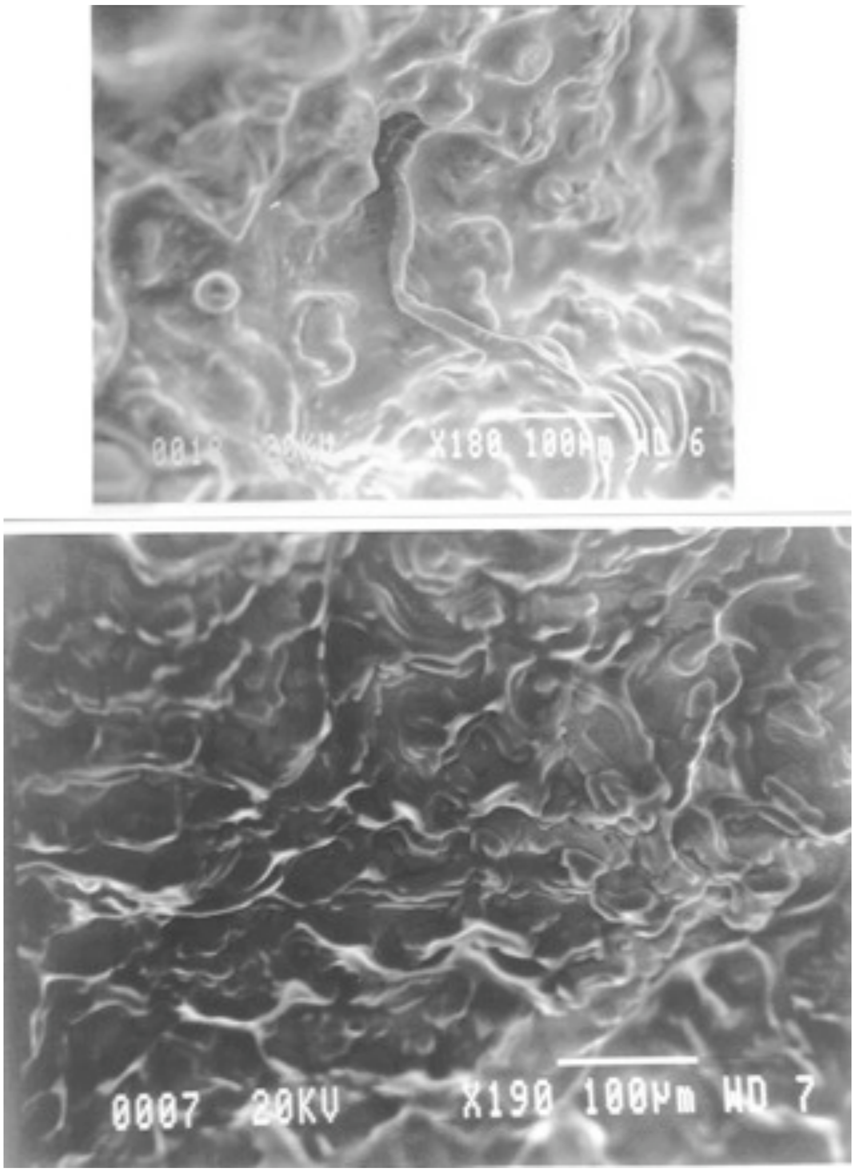

Figure 3 -- SEM picture. Application of the laser beam for 6 minutes shows that there are not round adipocytes; you only see fat liquefied 190Xmagnification times

4. *Without the use of tumescent solution, in vitro exposure of adipose tissue to laser beam for 4, and 6 minutes was performed and compared with samples without laser exposure (Zero minutes).* Laser penetration through adipose tissue decreased when the tumescent solution was not utilized. The scanning and transmission electron microscopic findings; after 6 minutes laser exposure, without the use of tumescent solution, correspond to those observed at 4 min. laser exposure by equal intensity (10 mW) combined with the use of a tumescent solution, suggesting that application of the tumescent solution is an important enhancement factor (Figure 4).

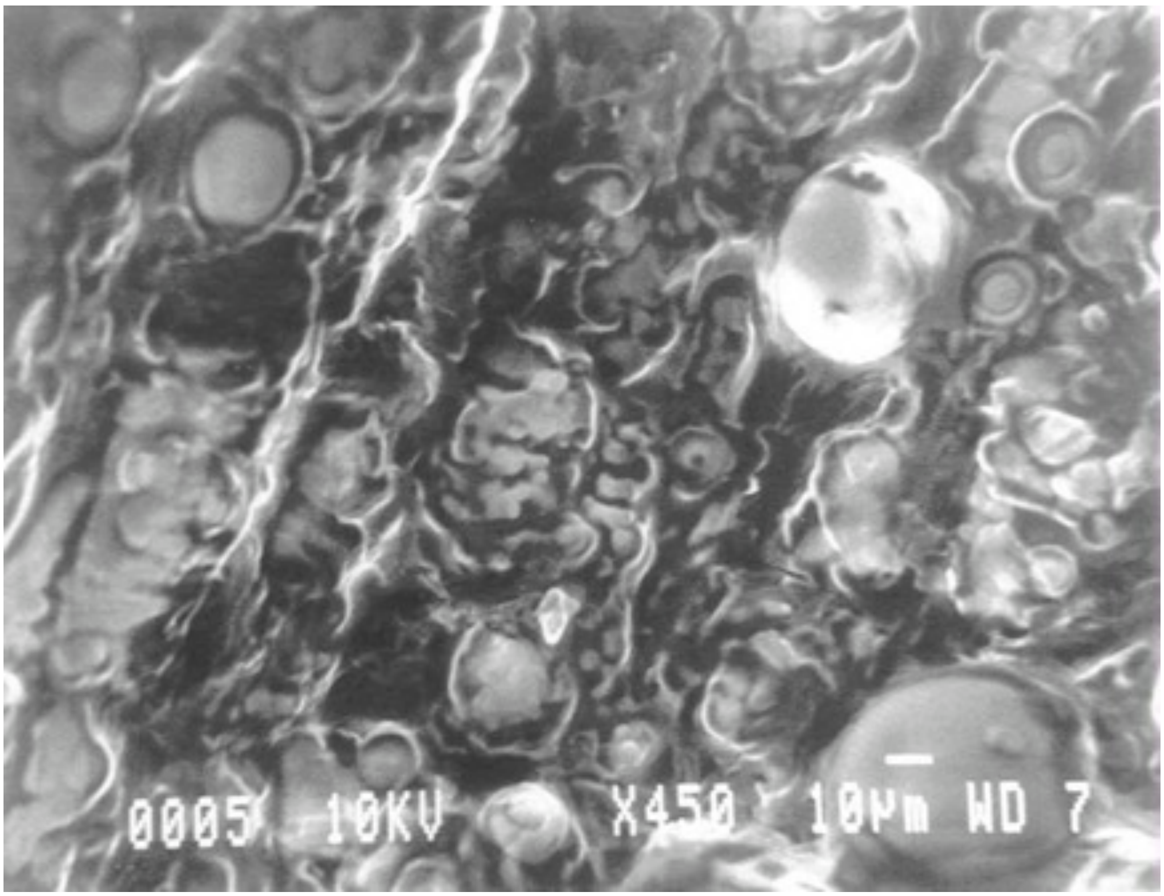

Figure 4 -- SEM picture. Application of the laser beam for 6 minutes without tumescence shows that some adipocytes are intact, but some are disrupted. 450X magnification times

5. Special effort was used in studying the membrane in order to clarify the suspected pore in the membrane.

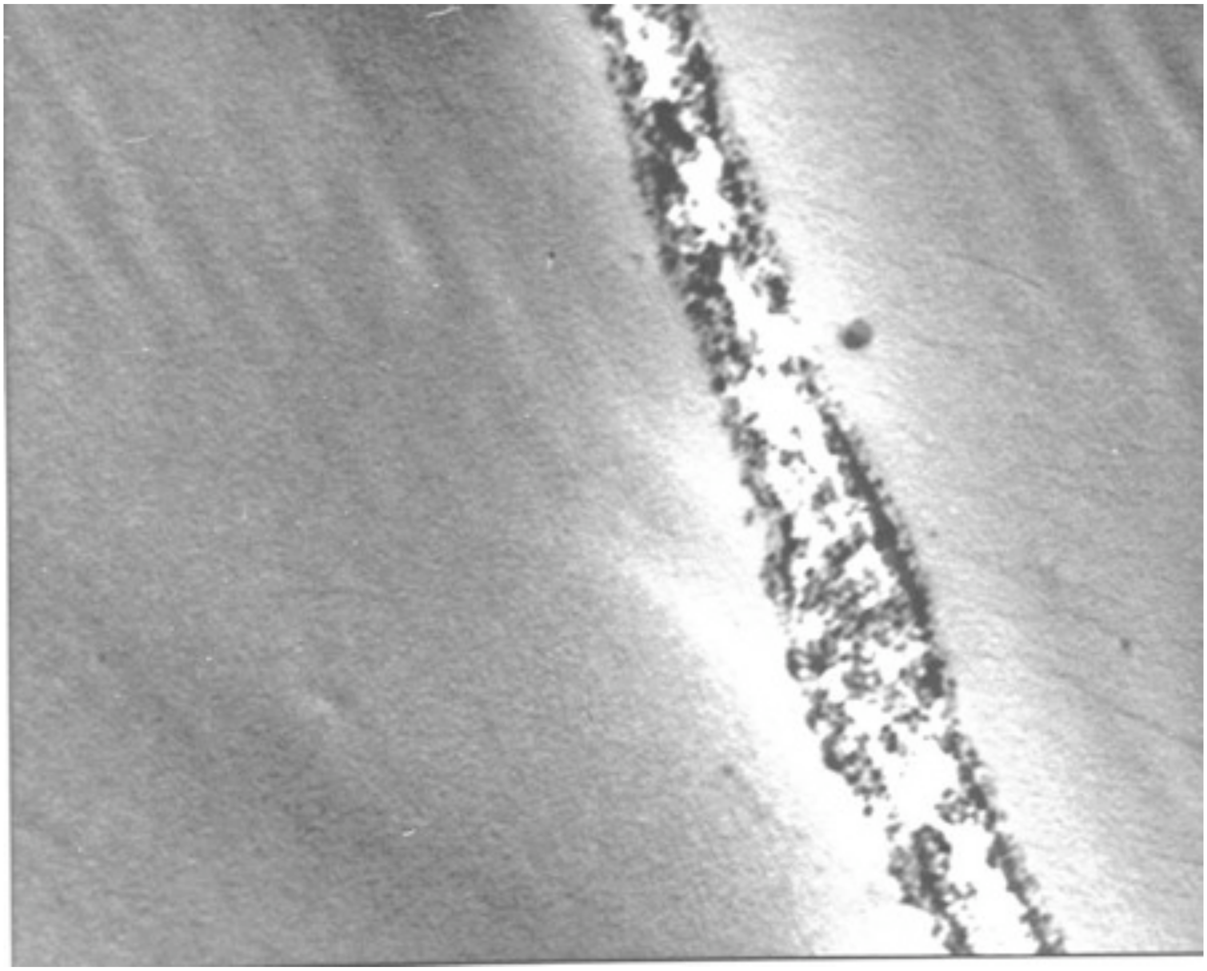

Figure 5 shows the adipose membrane at 40000X magnification – The membrane remains intact when the laser has not been applied.

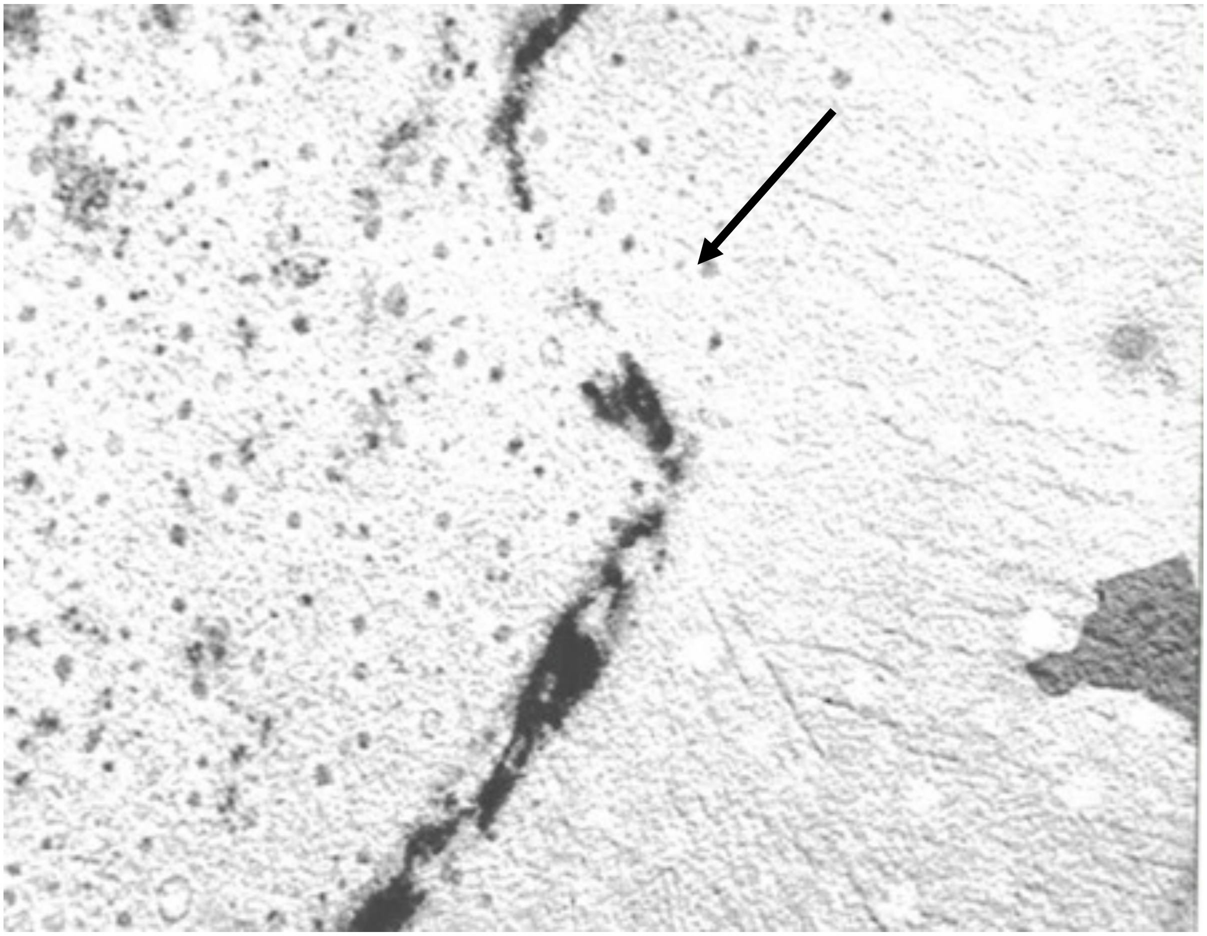

Figure 6 – TEM picture. Shows the cell membrane at 60000X magnification. After 6 minutes of laser exposure, the membrane is temporarily disrupted creating a transitory pore (see arrow) that allows the liquefied fat to come out of the cell and be released into the interstitial space.

In summary, at 0 minutes, without laser exposure the adipose tissue have its round shape (grape-like shape) (Fig 1). After 4 minutes laser exposure, the membrane of the adipocyte is partially disrupted and 80% fat is liquefied. Fat particles build up, forming a “cell helmet” (Figure 2A). Adipocytes suffer partial disruption of their membrane, exposing fat bodies within the cell (Figure 2B). At 6 minutes laser exposure, SEM shows almost total disruption of the adipose tissue (fat and cell membranes) (Figure 3).

Scanning and transmission electron microscopy verified the suspected Lipolysis. All possible scientific approaches have been applied resulting in the recording of over 180 pictures in order to demonstrate scientifically the technique described herein. To our knowledge, to this date, the use of the low-level laser energy to open a transitory pore in the adipose cell has not been reported. Therefore, the technique described below is a new application in the field of plastic surgery and this paper provides the scientific evidence to support to it.

DISCUSSION

Liposuction techniques and co-adjuvants have been used for many years. However, each time a new method or procedure appears, there are expectations about its potential benefits for mankind. The scientific evidence provided in this paper supports the L.A.L. technique and will serve as a valuable contribution to this specific field of medicine and generate the same expectations as other techniques previously described by other authors. Among its benefits are the reduced risk and improved quality of life for patients. Random samples taken from ten patients and submitted to SEM and TEM studies demonstrated that the application of the tumescent technique is an important co-adjuvant to laser beam application because it facilitates beam penetration and, as a result, fat extraction.

Findings were consistently observed as follows:

The SEM and TEM results indicate that 6 minutes exposure to laser beam with application of the L.A.L. technique and without tumescent technique were comparable to the recorded results achieved from 4 minutes laser beam with the application of the L.A.L. technique using tumescent technique. Theses findings support that tumescent technique therefore empowers the laser beam to extract fat from the cell.

Transitory pores were also observed in the cell membrane with the subsequent spillage of fat into the interstitial space.   In samples from tumescence technique, and without laser exposure, SEM shows that the adipocyte retained its original shape in this tri-dimensional picture. Several collagenic fibers can be observed in the interstice (Figure7).

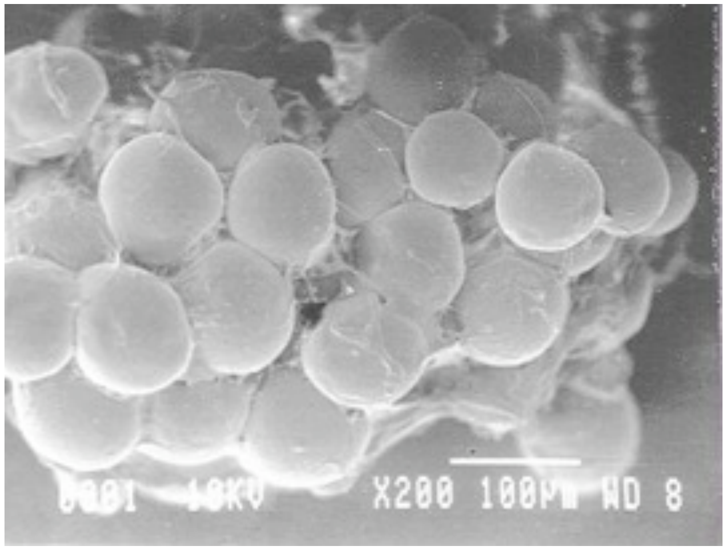

Figure 7 -- SEM picture. No laser exposure. Adipocyte intact, several collagenic fibers can be seen surrounding the adipose tissue. 200X magnification times

At 4 minutes laser exposure, without tumescent technique, liquefaction of only a few adipocytes occurred. (Figure 8) At 6 minutes laser exposure, without tumescent technique, SEM shows liquefaction of a higher number of adipocytes but not all.

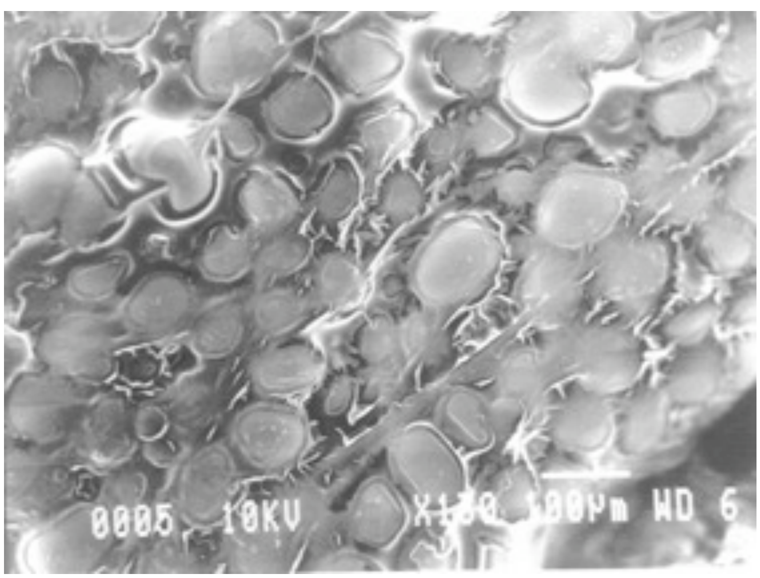

Figure 8 -- SEM picture. 4 minutes laser exposure. No tumescence has been applied. Only a few adipocytes have been liquefied. 190Xmagnification times

When the traditional tumescence technique was combined with a 4 minutes laser exposure, SEM shows (Figure 2A, 2B) partial disruption of the adipocyte membrane, this also shows how the cell membrane has been disrupted and 80% of the fat has been extracted from the cell (in the foreground). By increasing the laser exposure to 6 minutes, SEM shows almost total disruption of the adipocyte membrane. The cell membrane contour presents an irregular distribution. (Fig 3). In a similar way, for samples obtained from the traditional tumescence technique, without laser exposure, TEM shows adipocytes completely saturated with homogeneous fat. Figure 9 shows four of those cells, of regular diameter, close together and with a reduced intercellular space.

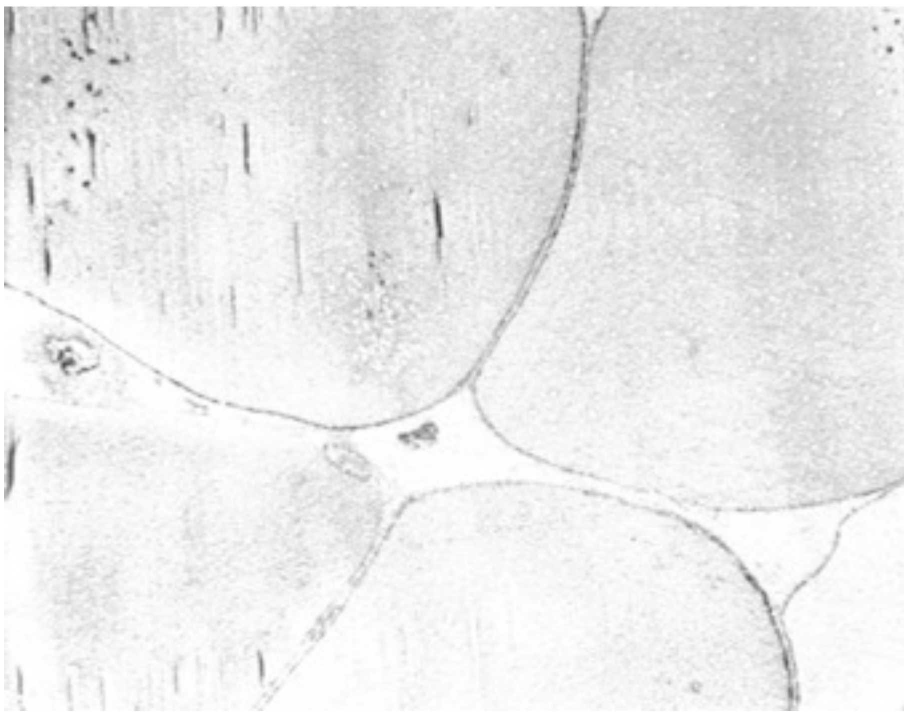

Figure 9 -- TEM pictures. The adipocytes are completely saturated with fat and close to one another. 20000 magnification times

When the L.A.L. technique was applied with a 4 minutes laser exposure, TEM shows partial loss of intracellular fat and increased intercellular space. (Figure 10) illustrates this fact and shows as well, three adipocytes. Deformed adipocytes that had lost their round shape were also observed. Capillaries remained intact with 4 and 6 minutes laser exposure (Figure 11).

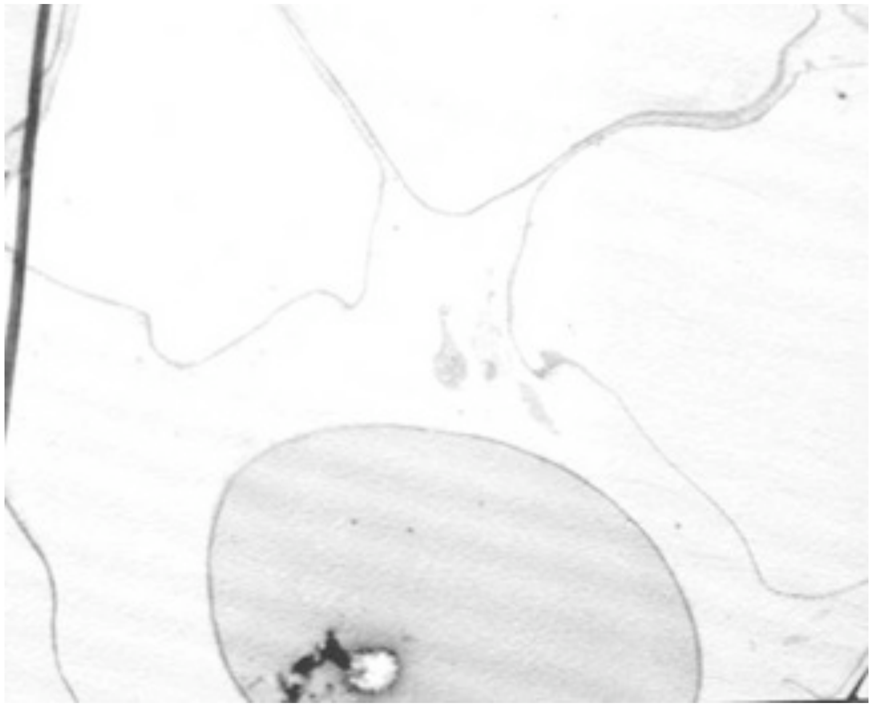

Figure 10 -- TEM picture. 4 minutes laser exposure. There is partial loss of the intracellular fat and the membrane has became flexed since has lost part of its fat content. 20000X magnification.

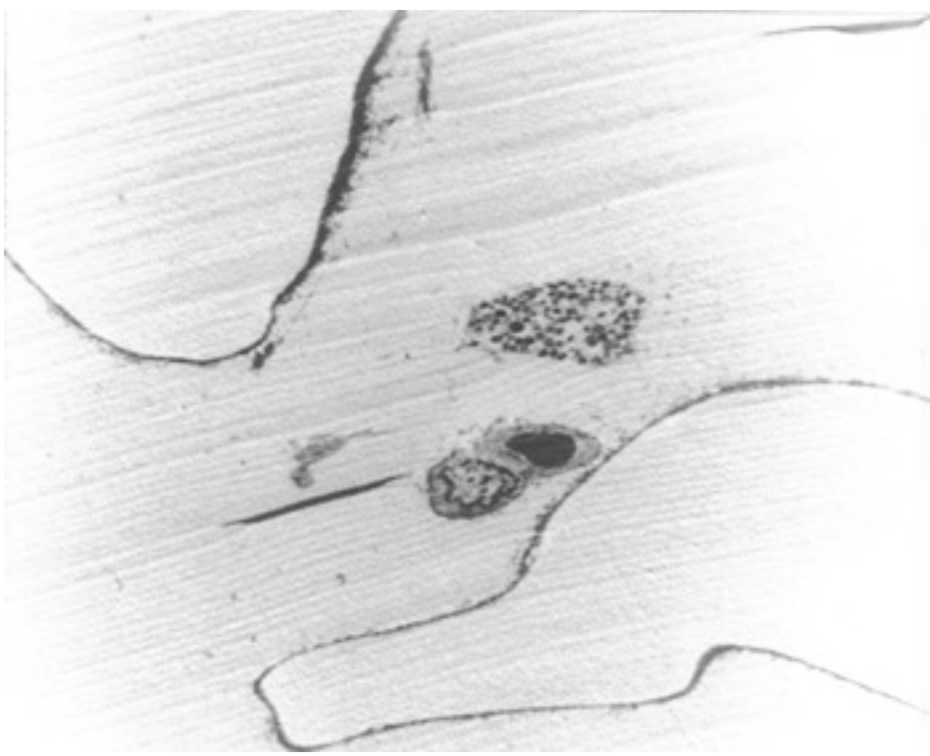

Figure 11 -- TEM picture 4 minutes laser exposure. The adipose membrane is flexed and deformed. Capillaries remain intact in the intercellular space. 20000X magnification.

When the L.A.L. technique was applied with a 6 minutes laser exposure, TEM shows almost total disruption of the regular contours of the adipocyte, intracellular fat is completely removed from the cell, and, therefore, the adipocyte is deformed and does not preserve its original shape (Figure 12). Figure 12 also shows deformity, folding, and disruption of the adipocyte membrane with 6 minutes laser exposure.

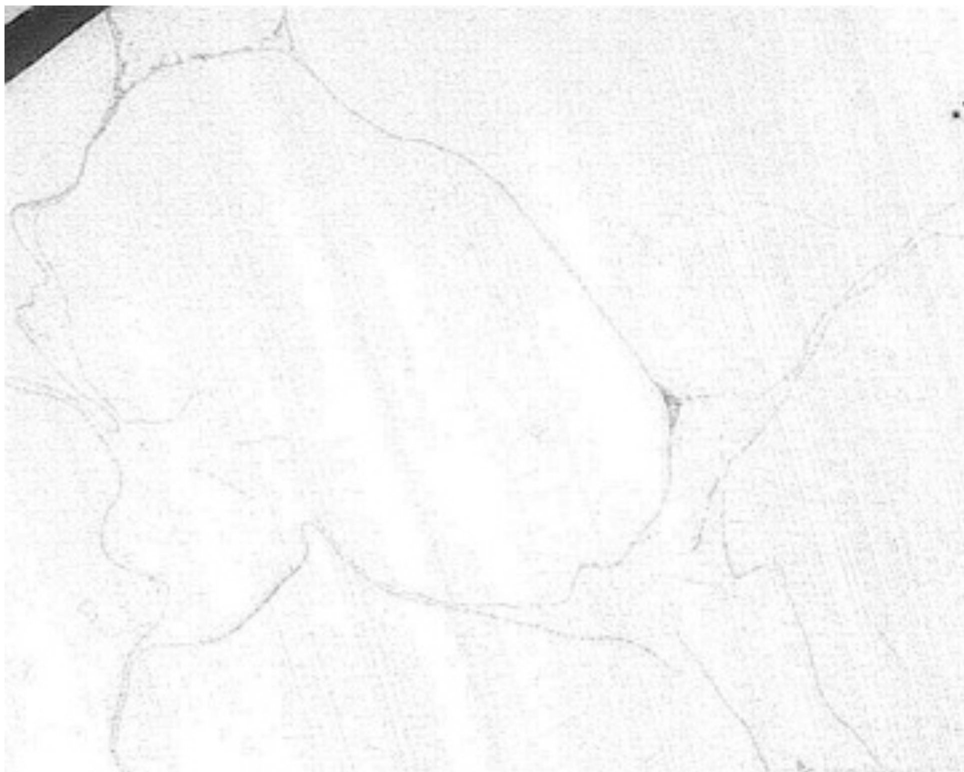

Figure 12 -- TEM picture. Recorded after 6 minutes laser exposure there is almost total disruption of the adipocyte membrane. The adipose cell has lost almost completely its fat content. 20000X magnification.

Interpretive explanation of our study findings, regarding the biological performance of the adipose tissue, its interaction with laser light, and the environmental contributions of the tumescent solution, experimental studies that show a 0.3 to 2.1% transmittance of red laser light in 2 mm thick normal skin, depends on the laser wavelength. Further, it was found that the transmittance of granular tissue is 2.5 times higher than that of the normal skin. Moreover, looking for a method to increase light transport deeply into target areas of tissue, the effects of a hyperosmotic agent on the scattering properties of rat and hamster skin were investigated (17), and a transient change in the optical properties of *in vitro* rat skin was found. A 50% increase in transmittance and a decrease in diffusive reflection occurred within 5 to 10 minutes after introducing glycerol. In our case, it is known that fat contains glycerol; therefore, laser transmittance through the adipocyte could be very effective. In addition, the tumescent solution has two mechanisms of action:

1. It is a polar solution that destabilizes the adipocyte membrane, thus facilitating the penetration of the laser beam. This was demonstrated by the findings in samples subjected to SEM and TEM.
2. The aqueous portion also serves as a co-adjuvant to laser action. These effects are co-adjuvants to the laser action, making the low-level energy laser a powerful tool in liposuction procedures.

The adipocyte membrane is activated by different AMPc concentrations, which stimulates, in turn, cytoplasmic lipase that triggers the conversion of triglycerides into fatty acids and glycerol, both elements that can easily pass through the cell membrane. The adrenaline, also found in the tumescent solution, stimulates the adenylciclase, which, together with the effect of the laser beam on the internal and external media of the adipocyte, change its molecular polarization. The exit and removal of fatty acids and glycerol into the extracellular space enhance this effect.

The effectiveness of low power laser light to produces changes in biological tissues and laser action on cells, even by low doses, is recently reported by different authors (17, 18, 19). Reproducible light-induced changes in the transmission spectrum of human venous blood under the action of low- intensity radiation from He-Ne laser was found (19), showing not only that laser light induces the changes, but the possibility of their spectrometric studies. Besides these, the influence of low-level laser irradiation on the mast cells degranulation process was preformed on mesentery mast cells of the rat thin intestine (20), showing that laser radiation (890 nm in this case) stimulates mesentery mast cells degranulation. This study shows also that the effect is dose dependent, and maximal degranulation was registered after laser irradiation with power of 25 mW, and an exposure time of 15-30 s. Finally, confocal microscopy was used for irradiation and simultaneous observation of low power laser effects in subcellular components and functions, at the single cell level (21,22). Cultures of human fetal foreskin fibroblasts (HFFF2) were prepared for *in vivo* microscopic. Cells were stimulated by the 647 nm line of the Argon- Krypton laser of the confocal microscope (0.1 mW/cm^2). Laser irradiation caused alkalization of the cytosolic pH_i and increase of the mitochondrial membrane potential. Temporary global Ca^{2+} responses were also observed. The effects were localized to the irradiated microscopic fields, and no toxic effects were observed, during experimentation.

CONCLUSIONS

The low-level laser assisted Lipoplasty (L.A.L.) consists of the tumescent liposuction technique with the external application of a cold laser (635 nm and 10mW in intensity, for a 6 minutes period). This technique produces a transitory pore in the adipocyte membrane, preserving the interstice, particularly the capillaries. When adipose tissue is exposed to the laser beam for 4 minutes, 80% of the adipocytes are disrupted; this increased to almost 100% with 6 minutes laser exposure demonstrated by scanning and transmission electron microscopy.

The laser facilitates the releasing of fat and contributes to the disruption of the fat panicles, allowing the fat to come from inside to outside the cell, placing it in the interstitial space.

The transitory pores formation induced by the laser occur exclusively at the level of the adipocyte membrane. When tumescent solution was used as co-adjuvant, almost 100% fat was released into the interstice, while capillaries and the remaining interstice were preserved. The result of this development is a safer, more effective procedure as the need for pre-tunneling has been eliminated.

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APPENDIX

Protocol to Identify Tissues by Scanning Electron Microscopy

1. Fix tissue in phosphate buffer with 2.5% 0.1M glutaraldehyde, at pH 7.2, for 24 hours.
2. Rinse buffer in 4.5% 0.1M sucrose phosphate buffer for 15 minutes.
3. Dehydrate in alcohol at different concentrations, 30%-100%, for 2 minutes per percentage alcohol.
4. Dry until critical point reached.
5. Place tissue on specimen holder previously prepared with colloidal graphite and attach with double adhesive tape.
6. Ionize with gold-palladium until a 10 to 18-monometer layer is formed.

7. Observe under the microscope; a JEOL-820 or JEOL-JEM 1010 scanning electron microscope can be used.

Protocol to Observe Ultrafine Tissues by Transmission Electron Microscopy

1. Fix tissue in milloning buffer with 2.5% glutaraldehyde at pH 7.
2. Rinse in 0.1M phosphate buffer at pH 7.2 for 15 minutes.
3. Postfix in 1% osmium tetra-oxide in distilled water for 1 hour.
4. Rinse in 0.1M phosphate buffer at pH 7.2 for 10 minutes.
5. Dehydrate in 25%-100% alcohol for 15 minutes.
6. Add 70% uranyl acetate to the alcohol during dehydration and leave tissues for 12 hours.
7. After last pass through 100% alcohol, pass three times through acetone for 15 minutes.
8. Infiltrate with 3:1 acetone + V plastic for 60 minutes.
9. Infiltrate with 2:2 acetone + V plastic for 60 minutes.
10. Infiltrate with 1:3 acetone + V plastic for 60 minutes.
11. Leave in pure plastic overnight.
12. Place in recently prepared plastic (SPURR).
13. Polymerize on stove at 60 °C from 8 to 15 hours.
14. Make ultrafine 600 nm-cuts with diamond-head scalpel in the ultramicrotome and collect in 1-hole grids covered with Fomvar membrane.

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