GREDECO
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EVALUATION OF THE i-LIPO™ DEVICE ON ANTI-CELLULITE EFFICIENCY USING A MODEL OF HUMAN SKIN EXPLANTS BY MEANS OF HISTOLOGICAL AND BIOCHEMICAL ANALYSIS

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I. INTRODUCTION

I-Lipo™ emits low levels of laser energy that disrupt the fat cell membranes, releasing cellular fat. This causes the cell to lose its round shape by changing the permeability of the cell membrane. This does not affect the neighboring structures such as skin, blood vessels, and peripheral nerves. Triglycerides spill out from the broken cell membranes and are released into the interstitial space, where they are slowly transported through the body's natural metabolic functions, with no adverse physiological effects, and are used by the body as an energy source. Light exercise post treatment can accelerate the breakdown and removal of fat from the zone of treatment.

Independent clinical studies have shown i-Lipo™ to be, in some cases, comparable to results achieved by liposuction. Ultrasound imagery shows up to 30% reduction in the fat layer depth after just one treatment. Additional treatments improve results further. Results can be seen immediately after each treatment as the fat cell contents are released. Light exercise post treatment can accelerate the removal of the released fat.

II. AIM OF THE STUDY

The aim of the study is to test the anti-cellulite activity of I-Lipo™ using an experimental model of human skin explants maintained in survival conditions. This alternative culture method avoids testing in-vivo biopsy specimens and maintains the model in near in-vivo metabolic condition. The direct application of the device on the skin model permits testing comparable to testing in-vivo.

This allows to visually and quantitatively measure the anti-cellulite effect using histological and biochemical analysis of the fatty tissue (hypodermis) comparing skin treated by i-Lipo™ device with untreated skin.

To evaluate the effect of the device, the main outcomes measured were:
- the **glycerol** level relevant to fat cells,
- the histological analysis of **fat cells and lobules**,
- the biochemical assay of **collagen synthesis** by the fibroblasts (to restructure the hypodermis after a decrease in the quantity of fatty tissue and to prevent relapse of cellulitis).

III. MATERIAL AND METHODS

1) Summary of our specific model of human skin sample maintained in survival conditions

A method of culture permitting the survival of normal skin in ex vivo conditions has been developed. This method has perfected past attempts meant to

keep skin samples in survival conditions (Chapman ,1989 ; Kondo,1990 ; Boisnic,1997a). The elapsed time for the procedure is artificially accelerated 3 times that of the normal time needed in vivo.

Skin fragments are cut into 3 cm^2 thick pieces and washed three times with an antibiotic solution (300 U/ml penicillin and 300 µg/ml streptomycin for the first washing procedure).

Skin biopsies are placed with the epithelium uppermost, at an air/liquid interface, on culture inserts (filter pore size 12 µm). These inserts are set on 6 well plates and culture medium was added underneath three times a week. Medium is Dulbecco's minimal essential medium (DMEM) containing antibiotics (100 U/ml penicillin and 100 µg/ml streptomycin), 200 µg/ml L-glutamine, bovine pituitary extract, growth factors and foetal calf serum. All supplements were freshly made at each medium change every two days. Skin biopsies are then put in a humidified atmosphere of 95% air-5% CO_2 at 37°C. This method provides for the testing of products or devices on the level of the epidermis.

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2) Method of treatment

In this protocol, skin fragments from 8 donors from patients undergoing plastic surgery are treated by 5 sessions (lasting 20 minutes each) of the iLipo™ every two days and then are maintained in survival condition with a humidified atmosphere of 95% air-5% CO_2 at 37°C. A comparison was made with untreated skin (control skin).

The supernatants were collected every 2 days for glycerol assay.

After 10 days of culture, one part of the skin was utilized for histological analysis. The remaining fragment was utilized for collagen synthesis.

IV. ANALYSIS

1) Histological evaluation of skin fragments

A comparison was conducted between the treated and untreated zones, consisting of an analysis of fat cells and an evaluation of the appearance of the hypodermis. Photography was used to visualize the obtained modifications between treated and untreated areas.

2) Glycerol released by the adipose tissue

The released glycerol in supernatants was assayed using the enzymatic method according to Vaughan (glycerokinase / glycerophosphate deshydrogenae). Results were expressed in μg glycerol / g of fat tissue.

3) Collagen synthesis

Skin fragments were enzymatically digested in an acetic acid 0.5M solution containing pepsin overnight at 4°C. The fibroblastic activity for collagen synthesis is evaluated by a spectrocolorimetric method (540 nm) measuring the acid-soluble new collagen synthesized after a specific fixation by Sirius red staining (Sircoll Collagen Assay, Interchim). The results were expressed in μg of collagen / mg protein.

4) Statistical analysis

Mean values and standard deviations were calculated for quantitative variables. Statistical significance was determined using the Student's t-test ($p < 0.05$ is considered to be statistically significant).

V. RESULTS

1) Histological evaluation of skin fragments

Figures 1 to 9 illustrated the anti-cellulite activity of i-Lipo™ device on the skin.

We analyzed changes in the hypodermis and adipocytes after i-Lipo™ treatment in comparison with control skin:

- Thinning of the hypodermis and reduction of fibrosis divisions between fat lobules;
- Small reduction of fat cell size with flatness or elongation instead of the roundness found in the adipose cells of control skin;
- Modification of fat cells' membranes with a withered appearance and occasionally a partial rupture of the cell wall;
- No necrosis or destruction of adjacent structures (epidermis, vessels, dermal collagen).

2) Glycerol released by adipose tissue

The results of released glycerol in supernantants were exposed in **Table I a** (mean ± ET) and in **Table I b** (individual results).

We observed a statistically significant increase of lipolysis in abdominal plasties following 5 treatment sessions with the i-Lipo™ device.

Glycerol levels increased to 233.5 µg/g fat tissue versus 177.16 for control skin ($p = 0.043$) (increase of about 30%).

3) Collagen synthesis

The results of collagen synthesis were exposed in **Table II a** (mean ± ET) and in **Table II b** (individual results).

An increase of collagen synthesis was observed after treatment by the i-Lipo™ device with a level of 46.2 μg/mg versus 40.25 for control skin (increase of 15%; $p = 0.001$).

VI. CONCLUSION

An anti-cellulite effect was observed in our ex-vivo human skin model after treatment by the i-Lipo™ device. A statistically significant increase in the amount of glycerol released by the adipose tissue was found, as well as a modification in the appearance of fat cells.

Moreover, a statistically significant stimulation of the dermal fibroblasts' metabolism was noted after i- Lipo™ treatment with an increase of collagen synthesis which acts to restructure the dermis and hypodermis following the shrinking of fatty tissue and also acts to prevent cellulitis relapse.

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Paris, France

Doctor Sylvie BOISNIC

Table I a:

Glycerol assay (μg glycerol / g fat tissue)

(mean ± ET, n = 8)

	μg /g
Control skin	**177.16** ± 78.7
Skin treated by i- Lipo™	**233.5** ± 99.3 * p = 0.043

***:** Statistical significant difference in comparison with control skin (paired Student's T test, $p < 0.05$)

Table I b:

Glycerol assay: individual results (μg/g)

	Control skin	Skin treated i- Lipo™
Skin 1	144.6	114.5
Skin 2	282	326.1
Skin 3	290	386.5
Skin 4	230.4	275.8
Skin 5	219.7	237.8
Skin 6	84.2	319.15
Skin 7	80.2	101.4
Skin 8	86.2	106.96

Table II a:

Biochemical assay of collagen (μg collagen / mg of skin biopsy)

(mean ± ET, n = 8)

	μg collagen / mg of skin biopsy
Control skin	**40.25** ± 11.6
Skin treated by i- Lipo™	**46.2** ± 9.3 * p = 0.001

***:** Statistical significant difference in comparison with control skin (paired Student's T test, $p < 0.05$)

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Table II b:

Biochemical assay of collagen: individual results (μg/mg)

	Control skin	**Skin treated i- Lipo™**
Skin 1	36.9	37.6
Skin 2	26.74	37.9
Skin 3	21.6	41.15
Skin 4	43.1	46.1
Skin 5	42.9	45.54
Skin 6	47.9	50.5
Skin 7	61.6	68.4
Skin 8	41.3	42.76