

ORIGINAL ARTICLE

Ex vivo human skin evaluation of localized fat reduction and anti-aging effect by TriPollar™ radio frequency treatments

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Abstract

Background: A wide variety of radio frequency (RF) treatments for localized fat and cellulite reduction as well as anti-aging are available nowadays, but only a few have shown the biological mechanism responsible for the clinical results. **Objective:** To determine the biological mechanism of the TriPollar™ RF device for localized fat and cellulite reduction as well as the collagen remodeling effect. **Methods:** Human skin samples were collected from abdominoplasty surgery and facial lifts, in order to evaluate the lipolytic and anti-aging effects of the apollo™ device powered by TriPollar RF technology using an ex vivo human skin model. The anti-cellulite effect was evaluated by the dosage of released glycerol and histological analysis of the hypodermis. Skin tightening was evaluated by morphometric analysis of collagen fibers and the dosage of collagen synthesis. **Results:** Following TriPollar treatment, a significant increase of glycerol release by skin samples was found. The structure of fat cells was altered in shape and a modification of the fibrous tract was also detected in the fat layer. Additional findings indicated stimulation of the dermal fibroblasts with increased collagen synthesis. **Conclusions:** The detected alteration in the hypodermal layer is manifested by fat and cellulite reduction accompanied by structural and biochemical improvement of dermal collagen, which result in overall skin tightening.

Key Words: Collagen, ex vivo, fat, radio frequency, TriPollar

Introduction

The pattern of fat distribution in the human body is affected by sex, diet, level of physical activity and mostly by genetics. One aesthetic problem for individuals who achieve modest or even dramatic weight loss due to a diet control program combined with physical exercise, is the inability to eliminate localized fat deposits from specific anatomical sites, such as the abdomen, buttock and thighs. These localized areas of fat accumulation cannot be solved by regular diet and require an additional body contouring treatment (1,2). Furthermore, this condition is usually found at specific areas of cellulite deposits, which is a common condition experienced by over 80% of postpubertal women (3).

Cellulite is characterized by an irregular dimpled configuration of the female adipose tissue, which is mainly found on body areas such as the thighs, buttocks and abdomen (4). It is caused by the enlargement of fat cells associated with modifications in the subcutaneous connective tissue (3,5).

A wide variety of topical preparations, invasive procedures and non-invasive treatments are being vastly used to improve the condition of cellulite. Recently, various non-invasive technologies, such as radio frequency (RF) alone, or combined with optical energy and/or with mechanical manipulations of the tissue, have been introduced for tissue tightening by volumetric heating of the superficial and deep layers (6,7). RF treatments have been accepted as one of the most popular and promising procedures for the treatment of cellulite, skin tightening and body sculpting (8–12).

The apollo™ device, powered by TriPollar™ technology, is a novel RF system indicated for the treatment of cellulite and for skin tightening. The technology is based on three or more electrodes designed to deliver a focused RF current into the dermal and hypodermal layers, generating heat through RF resistance. Focused and selective heating is intended to stimulate collagen remodeling and increase fat metabolism.

The biological mechanism of fat metabolism and collagen synthesis can be evaluated using an exclusive ex vivo human skin model (13–17). This model enables maintaining human skin fragments in survival conditions for a period of about 3 weeks while testing and analyzing specific parameters and skin characteristics.

The present ex vivo study is designed to explore the biological mechanism in which the apollo device powered by TriPollar RF technology affects fat cells and induces localized fat reduction simultaneously with collagen remodeling. Skin fragments harvested from abdominoplasty were used for fat metabolism evaluation whereas skin fragments from face lifts were used to evaluate the new apollo applicator dedicated to delicate and small treatment areas.

Material and methods

Maintenance of human skin fragments in survival medium

Human skin samples were obtained from eight abdominoplasty surgery procedures and four facial lifts in order to evaluate the lipolytic and anti-aging effects using an ex vivo human skin model. Skin biopsies were placed with the epithelium uppermost at an air/liquid interface on culture inserts (filter pore size 0.45 µm; Costar, Poly-Labo Paul Block, France). These inserts were set on a 12-well plate (Costar). Cohesion between skin and culture inserts was obtained with a polysiloxane vinyl seal in such a way that no skin retraction or lateral passage of any applied product towards the dermis was possible. Medium was added to the wells so that the surface of the medium was on the same level as the filter. Organ cultures were performed with Dulbecco's minimal essential medium (Gibco BRL, USA) containing antibiotics (100 U/ml penicillin and 100 µg/ml streptomycin; Gibco BRL), 200 µg/ml L-glutamine (Gibco BRL), bovine pituitary extract, growth factors and fetal calf serum (DAP, France). Skin fragments were kept in a sterilizer at 37°C and in an atmosphere of air/CO₂ (95% / 5%). This medium was renewed three times per week.

Evaluation of the lipolytic effect

Human skin fragments maintained in survival conditions

Skin fragments with hypodermis from abdominoplasty surgery ($n = 8$) were analyzed following a single treatment session with the apollo TriPollar device using the regular clinical protocol (skin temperature control, 5–10 minutes, power 50). A group of untreated samples was used as a control.

Skin fragments were kept in surviving conditions for 3 days. On day 3, skin fragments were collected for lipolysis evaluation by glycerol dosage measurement and histological analysis of hypodermis.

Glycerol level released by hypodermis of skin fragments

Hypodermis from the skin fragments was separated from dermis and placed in Krebs-Ringer bicarbonate buffer, pH 7.4, glucose 5.5 mM and bovine serum albumin 40 mg/ml. The preparations were shaken and the released glycerol was dosed by the enzymatic method according to Vaughan (18) (glycerokinase / glycerophosphate dehydrogenate). Results were expressed in nM/g of hypodermal tissue.

Histological analysis of hypodermis

A histological analysis was made of adipocytes and fibrous tract in the hypodermis after the treatment.

The following parameters were evaluated: the shape (inhomogeneity: elongated, irregular), the membrane (withered aspect, sometimes with partial rupture of the cell wall), as well as destruction or necrosis. A semi-quantitative measure was realized with scores of 1 to 4. The topography of these modifications in the hypodermis was also notified.

The modifications of fibrous tract were also examined: thinning or partial destruction of collagen fibers.

Evaluation of the anti-aging effect

Maintenance of human skin fragments in survival conditions and exposure to experimental aging model by UV

Four skin fragments collected from abdominoplasty surgery and four skin fragments from facial lifts were obtained.

UVA and B radiation, known to induce alterations in middle and profound dermis, was used to obtain premature aging of the skin with alteration of the dermis. The source of UV radiation was a Vilbert Lourmat stimulator (France) fitted out with a UVA irradiation source (365 nm) composed of tubes T-20.L-365 (no UVB, no UVC emission), mercury vapor tubes, low pressure, hot cathodes and then with a UVB irradiation source (312 nm) composed of tubes T-15.M-312 (no UVA, no UVC emission). The radiometer was associated with a microprocessor programmable in energy (J/cm²), with the time basis enabling six irradiation measurements per second for controlling the energy received by the skin fragment. One session with UVA 12 J/cm² and UVB 2 J/cm² was made.

Following radiation, a single treatment session with the apollo TriPollar device was performed, using

the regular clinical protocol (skin temperature control, 5–10 minutes at power 50 for abdominoplasty fragments and 4 minutes at power 15 for facial lift fragments). Then skin fragments were maintained in survival conditions for 12 days.

A comparison was made between:

- untreated skin
- skin aged by UV
- aged skin treated by TriPollar RF using the apollo device.

Collagen synthesis

Skin fragments were enzymatically digested overnight at 4°C in an acetic acid 0.5 M solution containing pepsin. The fibroblastic activity for collagen synthesis was evaluated by a spectro-colorimetric method (540 nm) measuring the acido-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 of skin.

Histological quantification of dermal collagen by computerized image analysis

Skin fragments were fixed in Bouin's solution (Formalin) and embedded in paraffin.

Serial sections of 4- μm thickness were obtained and specifically stained with a picric acid solution containing 0.1% sirius red.

For a quantitative analysis of these macromolecules, a computerized image analysis of each section was made. The stained slides were examined by a microscope (Leitz, magnification $\times 160$) connected with a camera unit (XC-75 CE type) and with a microprocessor (Q520).

The surface of the collagen bundles were measured in μm^2 . The relative collagen content of the dermis was then expressed as a percentage of surface analyzed dermis.

Statistical analysis

Mean values and standard deviations were calculated for quantitative variables.

The statistical significance of changes recorded with the measured parameters was determined with Student's *t*-test ($p < 0.05$ is considered to be statistically significant).

Results

Evaluation of lipolytic effect – glycerol dosage

Results are demonstrated in Figure 1.

A statistically significant increase of lipolysis in skin fragments obtained from abdominoplasty surgery

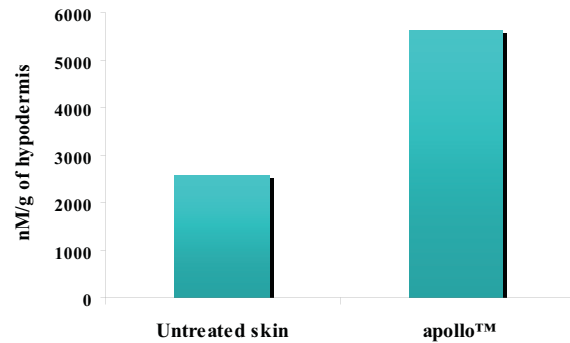


Figure 1. Glycerol dosage of apollo treated skin fragments versus untreated skin ($n = 8$).

($n = 8$) following a single treatment session with the apollo TriPollar device was found. The average glycerol level increased to 5610.2 nM/g hypodermal versus 2549.4 nM/g for untreated skin ($p = 0.045$), an increase of about 120%.

Histological analysis of the hypodermal layer

Modifications of the hypodermis were observed for two donors out of four. The histological aspect of adipocytes in the hypodermis after treatment demonstrated modifications in the shape (inhomogeneity: elongated, irregular) of the membrane (withered aspect, sometimes with the partial rupture of the cell wall). Modifications for donor no. 1 were the presence of a withered aspect of the adipocyte membranes on only one sector of the hypodermis. For donor no. 2, one necrotic sector of adipocytes in hypodermis was observed.

For all the donors, significant modifications were observed of the fibrous tract with thinning or partial destruction of collagen fibers (Figures 2 and 3).

Anti-aging effect

Collagen synthesis

A significant decrease of collagen synthesis was observed in the experimental skin aging model: $10.7 \pm 11.5 \mu\text{g}$ collagen/mg aged skin versus $14.1 \pm 12.0 \mu\text{g}$ collagen/mg for normal skin ($p = 0.02$).

Following the skin aging process, treatment with the apollo TriPollar (Figure 4) for the eight donors increased collagen synthesis with a level of $13.8 \pm 14.8 \mu\text{g}$ collagen/mg in treated skin versus $10.7 \pm 11.5 \mu\text{g}$ collagen/mg for control skin (an increase of 29%; $p = 0.02$).

For facial lift fragments, from only four donors, a more significant increase of collagen synthesis following treatment was observed: $5.2 \pm 2.4 \mu\text{g}$ collagen/mg skin versus $3.25 \pm 1.25 \mu\text{g}$ collagen/mg skin for untreated skin (an increase of 60%; $p = 0.03$).

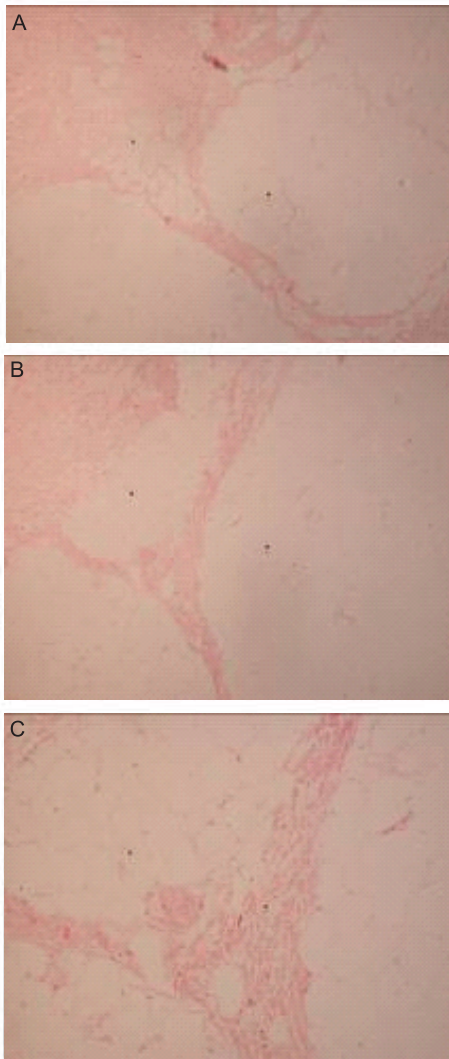


Figure 2. Hypodermis in untreated skin: (A) fibrous tract around adipose tissue (hematoxylin-eosin stain, $\times 40$); (B) thick fibrous tract around adipose tissue (hematoxylin-eosin stain, $\times 40$); (C) fibrous tract around adipose tissue (hematoxylin-eosin stain, $\times 100$).

(Figure 5). For abdominoplastic surgery, the increase was not significant (results obtained only from four donors).

Histological quantification of dermal collagen by computerized image analysis

UV radiations induced important alterations of collagen bundles. Histologically, these collagen bundles became thinner and disorganized in the dermis. Following apollo treatment, a dermal repair of collagen bundles was demonstrated, with the appearance of a dense collagen zone under the basal lamina (Figure 6).

Indeed, as shown in Figure 7, the surface occupied by collagen decreased in the superficial dermis

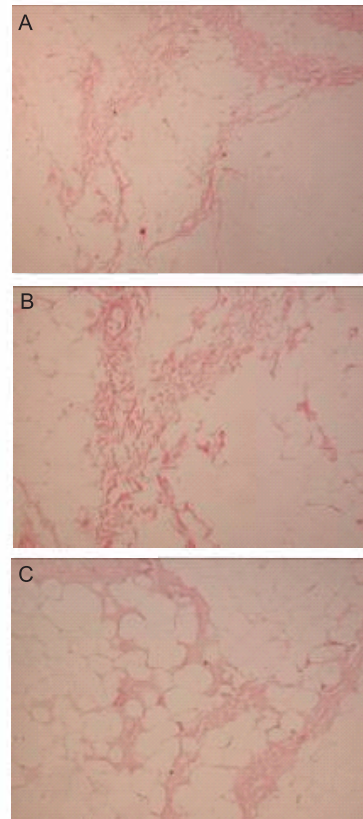


Figure 3. Hypodermis after treatment with the apollo TriPollar RF device: (A) modification of fibrous tract around adipose tissue with partial destruction of collagen bundles (hematoxylin-eosin stain, $\times 40$); (B) partial destruction of collagen bundles with alteration of adipocytes (hematoxylin-eosin stain, $\times 100$); (C) thinning of fibrous tract of hypodermis with partial destruction of adipocytes (hematoxylin-eosin stain, $\times 100$).

after UV ($53.13 \pm 16.8\%$) in comparison with normal skin ($66.25 \pm 17.8\%$) ($p = 0.001$) and also in the mid-dermis ($56.2 \pm 15.5\%$) versus normal skin ($69.75 \pm 13.0\%$) ($p = 0.006$). After treatment of the eight donors with the apollo TriPollar device, a statistically significant collagen repair was observed with $60.6 \pm 12.0\%$ of collagen in the superficial dermis versus $53.13 \pm 16.3\%$ for control skin (increase of 14%; $p = 0.045$), and with $66.9 \pm 10.9\%$ of

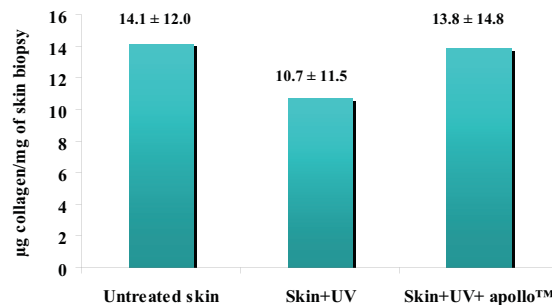


Figure 4. Biochemical dosage of collagen from abdominal and facial skin samples.

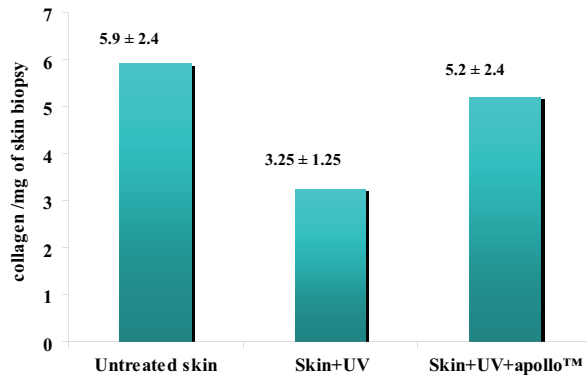


Figure 5. Biochemical dosage of collagen from facial lift surgery.

collagen in the mid-dermis versus $56.2 \pm 15.5\%$ for control skin (increase of 19%; $p = 0.006$).

From facial lift only donations (Figure 8), we also observed a significant increase of collagen with $51.9 \pm 8.8\%$ in the superficial dermis versus $40.36 \pm 14.0\%$ for untreated aged skin (increase of 28%; $p = 0.03$) and with $58.4 \pm 9.2\%$ in the mid-dermis versus $45.4 \pm 9.8\%$ for untreated aged skin (increase of 28%; $p = 0.005$).

Discussion

The biological basis of non-invasive fat reduction and collagen remodeling has been extensively investigated in recent years. Clinical and histopathological studies were conducted to evaluate the effects of various RF-based technologies on dermal and hypodermal skin layers. Various processes have been suggested to occur following selective electro-heating of the dermal and subcutaneous layers, including stimulation of fat metabolism, an increase in local circulation and lymphatic drainage of adipose tissue (9,12,19), volumetric contraction of connective tissue in the subcutaneous layer (20) and skin tightening effects resulting from the formation of new collagen fibers (10,20).

Emilia Del Pino and colleagues (20) evaluated the effect of monopolar RF on cellulite and subcutaneous tissue of the buttocks and thighs. Two RF treatments spaced 15 days apart were administered on 26 healthy female patients with visible bilateral cellulite (grades I–III) on either the buttocks or the thighs. The distance between the dermis and the Camper's fascia was measured using real-time scanning ultrasound imaging. Results demonstrated that following two treatment sessions at an interval of 15 days, the thickness measured between the dermis and fascia was shortened, with an average reduction of 2.64 mm and 1.8 mm at the thigh and the buttock, respectively. When analyzing the changes in the Camper's fascia between the first session and 45 days later, they repeatedly observed a noticeable organization of the fibrous lines, as well as an increase of the fibrous tissues in 53% of the cases, and an increase of the thickness of the fibers in 57% of the cases.

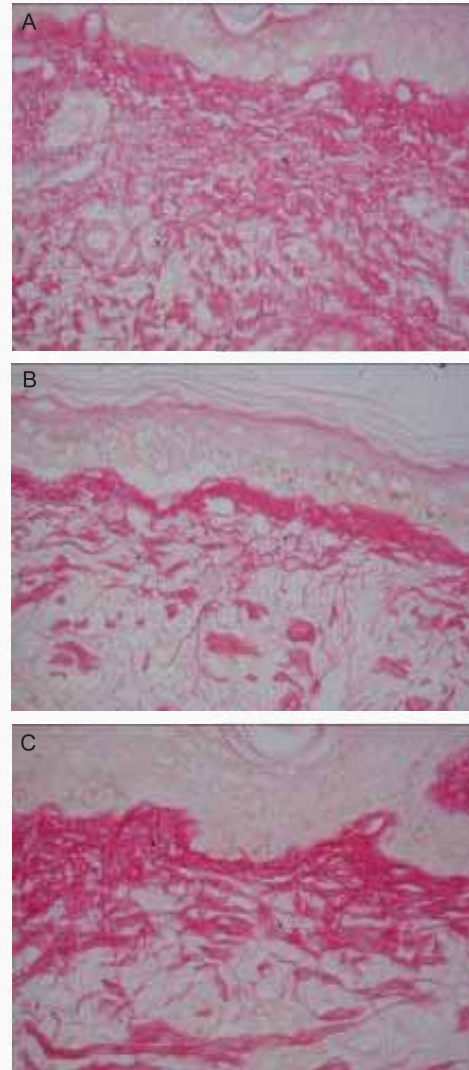


Figure 6. Collagen tissue or skin fragment from facial lift: (A) untreated skin (sirius red X400); (B) skin + UV: alteration of dermis with destruction of collagen bundles (sirius red X400); (C) skin + UV + apollo: repair of collagen bundles in dermis with appearance of dense zone under basal lamina (sirius red X400).

Most patients were satisfied with the results; the most satisfied were the women who had the most accentuated defects.

Another report by Goldberg et al. (10) demonstrated clinical, histological and MRI analysis of cellulite treatment with a unipolar RF device. A total of 30 subjects with upper thigh cellulite were treated every other week for a total of six treatments. An epidermal skin temperature of 40–42°C was maintained during treatment. Twenty-seven subjects showed evidence of clinical improvement with a mean decrease in leg circumference of 2.45 cm. Histologies demonstrated dermal fibrous band thickening. In this study, no changes in the pannicular layer, including Camper's fascia, were noted at the 6-month follow-up.

Montesi (21) reported clinical and histological results using a bipolar RF device combined with

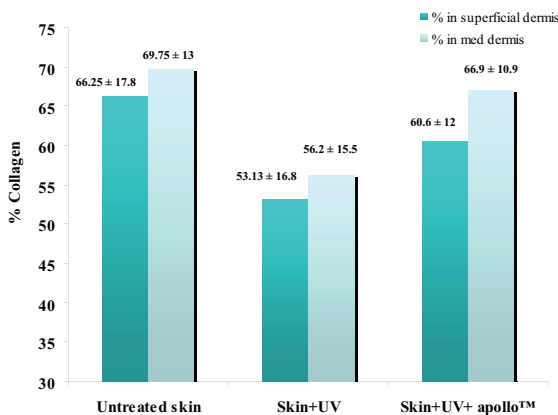


Figure 7. Histological and morphometrical analysis of collagen bundles from abdominal and facial skin samples (sirius red staining).

vacuum for the treatment of wrinkles, skin laxity, acne scars and striae distensae. Thirty patients underwent a cycle of six to eight sessions with 2-week intervals and the results were monitored photographically. In addition, 15 patients were subjected to two biopsies: one at the start of treatment and the other 3 months following the last treatment. All patients showed improvement in treated imperfections from the second session onwards and expressed satisfaction at the end of the treatment cycle. The most notable results were observed in striae distensae and 3-mm punch biopsies confirmed the clinical results observed. Biopsies of untreated skin showed atrophic and intensely elastotic dermal collagen, while treated skin showed a decrease in collagen atrophy as well as an increase in interstitial edema, indicative of better dermal trophism. In biopsies performed on the striae, a marked decrease in sclerosis together with an increase in the organization of collagen fibers was observed.

The ex vivo method of long-term skin culture used in this work presents numerous advantages (13,17). This technique mimics the in vivo environment; human biopsies can be avoided and animals are spared. Final formulation of a product or medical device can be applied directly to the epidermis, reproducing the exact conditions of in vivo use. By optimizing long-term organ culture conditions, viability and a fully differentiated state of normal human skin have been maintained ex vivo.

The current ex vivo study was intended to evaluate the biological basis for fat and cellulite reduction and skin tightening effects of the apollo device powered by TriPollar RF technology. The evaluation was done ex vivo using human skin fragments harvested from abdominoplasty and face lifts which were maintained in survival conditions.

As RF selectively heats fat cells to increase natural fat metabolism and secretion of liquid fat, the anti-cellulite effect was evaluated by dosage of released glycerol and histological analysis of hypo-

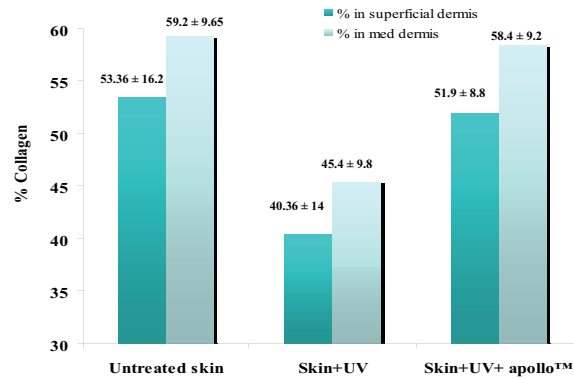


Figure 8. Histological and morphometrical analysis of collagen bundles from facial lift surgery (sirius red staining).

dermis. The tightening of the skin was analyzed by morphometric analysis of collagen fibers and dosage of collagen synthesis.

Lipolytic activity was observed in the ex vivo human skin model as measured in skin fragments harvested from abdominoplasty surgery and treated by the apollo TriPollar device. A statistically significant increase in the amount of glycerol released by adipose tissue was measured following treatment. The study results also demonstrated a statistically significant stimulation of the dermal fibroblasts and increased collagen synthesis following TriPollar RF treatment by the apollo device. A statistically significant repair of altered collagen in the eight skin fragments was observed, with a greater level of repair on fragments collected from facial lift surgery as compared with skin fragments from abdominoplasty surgery.

In summary, the effects observed after treatment with the apollo device powered by TriPollar RF technology confirmed and further expanded previous clinical results (17).

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Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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