

Non-Surgical Body Contouring: Introduction of a New Non-Invasive Device for Long-Term Localized Fat Reduction and Cellulite Improvement Using Controlled, Suction Coupled, Radiofrequency Heating and High Voltage Ultra-Short Electrical Pulses

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Abstract

Objective: To evaluate the safety and efficacy of a new and novel radiofrequency (RF) device used for focal fat reduction.

Materials and methods: 24 female and 1 male patients were enrolled in the study. The age range of the patients was 28-62 years old and an average BMI was 26. All patients underwent focal shape correction, without anticipating any weight reduction and 14/24 patients also had posterior or anterior grade 2 or 3 thigh cellulite treated. The patients underwent a 6-treatment protocol where they were treated with a novel RF device on the abdominal and flank regions once a week over a period of 6 weeks. 14 patients also had cellulite on their posterior or anterior thigh treated with the same device and a 6-treatment, once weekly protocol. Therapeutic thermal end points for each treatment were established and achieved as outlined in the paper. The RF device emits both basic RF and ultra-short High Voltage Pulsing (HVP). All patients and zones were treated once per week for 6 weeks.

Results and conclusions: All patients were observed for 3 months following their last treatment to determine the long-term adipose tissue effects and body contour changes. Standard pre and post-operative photography, circumferential measurements were taken. 3 patients who were scheduled for future abdominoplasty surgery had Investigational Review Board compliant lower abdominal subcutaneous biopsies performed at 72 hours and 14 days following their initial RF treatment. The average circumferential reduction was 3.58 cm with range of 1.5 cm-4.4 cm. There were no non-responders observed in the study. Using the modified Nurnberger-Muller 7 stage cellulite grading system, the average cellulite score improved 2 sub-grades. Using the Vectra 3D imaging device and cellulite scoring software, the average starting pit depth was 4.1 mm (range 0.5 mm-7.3 mm). Average Vectra measured improvement in the pit depth or "smoothness score" was 2.9 mm or 60% (range 1.1 mm-6.3 mm). Biopsies revealed histological evidence of the death of proportion fat cells by apoptosis. There were no complications that required medical management. We conclude that the TiteFX appears to offer a safe and effective option for the non-invasive management of both focal fat excess and cellulite. The basic RF and High Voltage pulses appear to result in a long-term, proportional death of fat tissue, presumably through adipocyte apoptosis.

Keywords: Fat reduction; Cellulite improvement; Radiofrequency; TiteFX; Electroporation; Adipocyte apoptosis; Ultra-short High Voltage RF Pulsing

Introduction

Ablative therapy for neoplasm, both benign and malignant has been deployed as an alternative for extirpative surgery for many years [1]. Reported ablative therapies include low temperature cryotherapy, focused ultrasound, intra-lesional chemical ablation, interstitial laser therapy and radiofrequency ablation or RFA [1]. Bio-electrics is a relatively new player in ablative therapy and apply pulsed, high voltage electrical currents to cell biology, specifically cell membrane physiology [2-4].

The integrity and cellular functions of a mammalian cell are, in part, maintained by trans-membrane potentials that are controlled by complex cell membrane functions. Exposing a cell to specific pulsed electrical currents can alter the trans-membrane potential of mammalian cells and, if the duration of the applied electrical pulses are less than the "charging time" of the outer cell membrane (approximately 100 nanoseconds), there is a non-thermal interaction with both the cell membrane and sub-cellular structures [5]. By manipulating the pulse duration (creating ultra-short pulses), electric field intensity, and a train, or pulsed sequence of these pulses, it is possible to alter the cell membrane and affect cell survival [5]. Specifically tailored pulsed

electric fields have the ability to increase the permeability of mammalian cells, a process known as electroporation, which can be "reversible" or "irreversible". Reversible electroporation has been widely deployed in medicine to facilitate non-permeable chemicals to cross cell membranes [6]. In practice, a non-permeable chemical is introduced in the body and a direct current is applied to the target tissue and cells, typically, a sequence of eight, 100 microsecond pulses, with up to 1000 volts, each pulse separated by 100 millisecond pulse delays are delivered [6]. Reversible electroporation and trans-membrane chemical substance delivery have had applications in cancer and gene therapy [6]. Ultra-short nanosecond pulses have demonstrated direct, non-membrane

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intracellular structures, organelles and the nucleus with applications in cancer, platelet function and gene therapy [2-4].

Irreversible electroporation (IRE) delivers a tailored electrical pulse configuration specific to the target cell and permanently alters cell permeability, resulting in an influx of ions, and altering the trans-membrane potential and intracellular function such that the target cells stop functioning and dies over time. Industrial applications have included water sterilization, where the IRE targets and kills bacterial and yeast cells [7]. More recently, IRE has been shown to ablate tumor cells, both *in vitro* and *in vivo* in mammalian animal studies [7-9]. The therapeutic advantages of IRE in treating tumors are the non-thermal mechanism and the relative sparing of surrounding structures. By deploying IRE, the cell membrane electroporation thresholds are different from the tailored IRE pulsed electric profiles used on the tumor tissue, creating selectivity [7-10]. Pre-heating of tissue prior to the train of ultra-short, high voltage pulses appears to reduce the electroporation threshold and enhance the efficacy of producing IRE [11]. To date, we are not aware of any published studies on high voltage, ultra-short, pulsed sequenced IRE on adipocytes. This study analyzes the cellular effects of a novel basic Radiofrequency and ultra-short, high voltage pulsed system on adipose tissue and the aesthetic body contour changes that may result.

Non-invasive body contouring is one of the fastest growing market segments in aesthetic medicine [12,13]. Non-invasive body contouring technologies have progressed from non-thermal, mechanical rollers and suction systems used in the 1990's, that enhanced lymphatic drainage in cellulite and fat deposits, to thermal based devices which combined laser, radio-frequency (RF), hypo- or hyperthermia and/or infrared energies with or without mechanical massage and/or suction [14-27]. The most common optical energy sources used in body contouring devices have been in the near and far Infrared (IR) spectrum. These optical, IR or RF systems, with or without rollers, with or without suction, deliver a thermal stimulus to the superficial adipose tissue to enhance normal lipolysis metabolism. In addition, these devices decrease, by a reduction of the triglyceride cytosolic volume, without cell destruction, the adipocyte volume (conversion of triglycerides to glycerol and free fatty acids which egress out of the cell and are mobilized and metabolized through normal hepatic pathways), through thermal stimulation of epinephrine mediated membrane bound lipase [13,14,27]. Infrared energy penetrates into the dermis, leading to a modest tightening effect on the skin and some improvement in cellulite, while RF devices, depending upon the system, can penetrate 5-25 mm into the subcutaneous tissue affording the opportunity to have a more direct effect on adipose tissue. Since the earliest RF non-invasive body contouring systems, the VelaSmooth and VelaShape which are suction couple IR, RF (Syneron Ltd, Israel) and Thermage (Solta Medica, USA), there has been proliferation of RF systems with moving mono-polar, bi-polar, tri-polar and octi-polar electrode applicators. These thermal RF and optical systems are "non-disruptive" in that the adipocyte cell membrane and cellular functions are not damaged. Despite being temporary "metabolic enhancers" increasing physiological breakdown of triglyceride, most RF systems for non-surgical body contouring report 2-4 cm circumferential reductions in abdominal-hip measurements, 2-3 cm in thigh reduction, with an average 20-60% improvement in the appearance of cellulite and, when well selected, consistently happy patients [14-27]. However, the disadvantages of these "non-disruptive" body contour and cellulite systems is the transient effect on adipose tissue and the contour enhancements that may be achieved [14-27].

Over the past few years there have been several non-invasive body contouring technologies that have come to the market that do disrupt and permanently damage the adipocyte and provide the potential for long-term focal fat reduction and body contour improvements. These "disruptive technologies" damage and permanently injure the fat cell through: [28-42]

- (i) Cavitation (the Ultra shape focused, high frequency ultrasound, or HIFU)
- (ii) Thermal disruption (Liposonix high frequency focused ultrasound)
- (iii) Freeze the fat cell and induce an adipocyte apoptosis through cryolipolysis (Zeltiq)
- (iv) The creation of a temporary "pore" in the adipocyte cell membrane for triglyceride egress (Zerona)

The Ultra shape uses gentle, non-thermal ultrasound waves to induce a cavitation disruption of the cytosolic fat droplets and "non-thermal implosion" of the adipocyte. The Ultra shape protocol generally calls for 3 treatments, one every two weeks and with the new vertical depth focus ultrasound (simultaneous ultrasound treatments at three different layers) and RF applicators, abdominal circumferential reductions 5-6 cm have been reported after 6 weeks. Advantages of the Ultra shape are a virtually essentially painless treatment, ease of use with automated tracking systems for the ultrasound and minimal treatment edema so results can be seen within a short time. Ultra shape disadvantages are principally the disposable ultrasound pulse costs [28-35].

Liposonix uses a thermal HIFU approach and multiple passes to induce layered adipose thermal necrosis. Liposonix purports a single treatment protocol, but disadvantages include reports of significant patient pain (necessitating benzodiazapines and narcotics), ecchymosis, and edema and swelling following the adipose thermal necrosis, so often, results are not visible for up to 3-6 months due to gradual remodeling of the intense thermal necrosis. Circumferential abdominal reductions of 3-6 cm have been reported using Liposonix after a single session. Liposonix also has a pulses disposable cost [36,37].

Zeltiq's "Coolsculpt" uses suction coupled "super-cooling" applicators applied to the flanks and abdomen. The patient and the device are left for between 1-4 hours (an hour for each love handle and one for the lower abdomen). The advantages of cryolipolysis are that it does not need a technician to perform the procedure and patients can be left in a side office and the treatment is relatively comfortable. Single treatment protocols have shown up to 20% of the adipose between the treatments paddles undergo apoptosis death resulting in a 3-6 cm average in abdominal circumferential reduction after a single session, but results are not completed for 4-6 months following the adipocyte apoptosis. Disadvantages include limited treatment zones as areas other than the love handle or lower abdomen (you must be able to grab and retract the adipose tissue and skin) cannot be currently treated (although new applicators are planned), there is also a high disposable cost and there have been reports of painful dysaesthesias for several months after treatment, presumably from a hypothermic injury of the sensory nerve lipid membrane [38-40,13].

Zerona uses an array of 650 mn diodes strategically placed just above the skin to induce a cytochrome oxidase induced conformational alteration in the adipocyte bi-lipid cell membrane and the transient "pore". It is through this temporary "fat pore" that triglycerides can migrate, resulting in a reduction in the adipocyte size and hence adipose tissue reduction. The protocol for this painless Zerona

procedure can also be performed in a side office, without a technician and involves 6-12 treatment protocols every second day for 2-3 weeks. Disadvantages include a relatively modest incidence of non-responders and a disposable cost [41,42,13].

All of the aforementioned “disruptive” fat contouring technologies have a disposable component and they are not designed, nor are there any prospective reports of cellulite reduction.

Although the etiology of cellulite is not completely understood, there are hereditary, hormonal and perhaps other metabolic multifactorial influencers. It is estimated that 70% of post pubertal women have cellulite and it can be quite independent of BMI, as very thin woman can develop it and quite obese women may not [13,14-27]. The clinical phenotypic appearance of cellulite is well known, the “lumpy-bumpy, cottage-cheese” or “orange peel” appearance of the skin. Anatomically, cellulite occurs most commonly on the posterior thighs and buttocks, followed by lateral thighs, anterior thighs and upper abdomen. Cellulite is commonly classified by the modified 7 stage Nurnberger-Muller score [34], according to severity (Table 1). Histologically, cellulite is characterized by the following [14-27]:

- (i) Edematous, swollen lobules of superficial adipose tissue with poor microcirculation and low tissue oxygenation
- (ii) Herniation of the swollen lobule of fat up into the deep reticular dermis and forming the “nodules”
- (iii) Dense, thickened vertical fibrous septae that are congealed, contracted extensions of the oblique and vertical intra-lobular septae and result in dermal retraction or “pits”

Although the exact cause of cellulite may be obscure, like the focal fat reduction technology, many of the RF, suction and massage devices discussed above have shown some variable improvement in the appearance of cellulite, with average improvements of 20-70% after a series of treatments [13,14-27].

The desirable features of a non-invasive body contouring, fat reduction and cellulite improvement device include [13]:

- (i) Some energy mediated thermal, chemical or electrophysiological effect on the adipocyte that destroys the cell permanently, or incapacitates the cellular function leading to apoptosis (cell death). The final pathway can be thermal (high temperature or very low temperature), cavitation influences or lipid bi-layer membrane disruption. The advantage of a “disruptive” technology is a long-term body contouring and cellulite reduction
- (ii) High degree of safety
- (iii) Minimal discomfort
- (iii) High percentage of adipose tissue impacted
- (iv) High degree of efficacy and reproducibility
- (v) Disruptive effects on the adipocyte for long lasting body contour and cellulite effects
- (vi) Impacts the appearance of both focal fat and cellulite and can be used on multiple potential body areas
- (vi) Revenue efficient with low or no disposable costs

The objective of this study is to assess the safety, efficacy and possible mechanism of a novel basic RF and high voltage, ultra-short pulsed device on the treatment of both focal fat collections and cellulite. This paper reports a novel technology that represents a new

body contouring device, the TiteFX (Invasix, Israel) which offers many of the ideal body contouring features patients and physicians seek.

Materials and Methods

The TiteFX device (Invasix Ltd.) combines, in a synergistic fashion, the following suction coupled therapeutic energy sources:

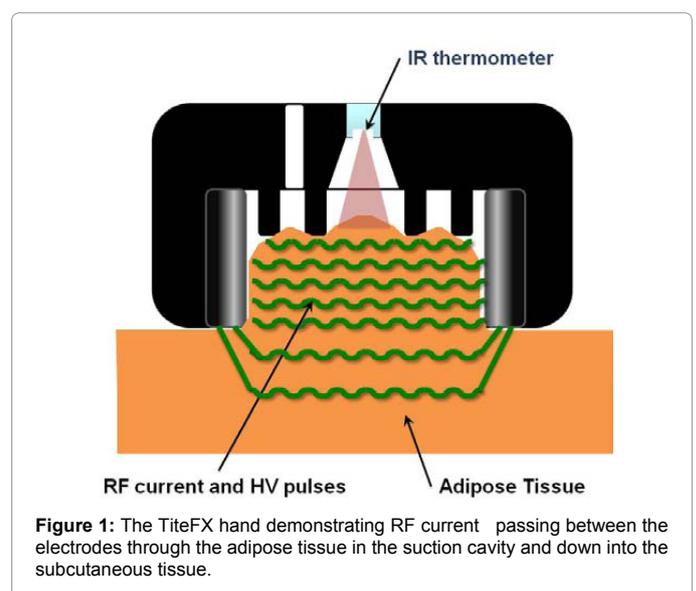
- Uniform RF heating of the skin and subcutaneous fat with real time monitoring of skin temperature using an infrared thermometer built into the hand piece and a built in “on-line” temperature cut-off feature that prevents overheating, painful hotspots and ensures prolonged thermal uniformity at the desired end-points.
- High-voltage (HV), ultra-short RF pulses applied to the subcutaneous fat are designed to deliver irreversible electroporation (IRE) effects to the adipocyte leading to delayed apoptosis of the fat cell and adipocyte cell death. These HV pulse electroporation effects on cell membranes can be seen in other tissues.

The TiteFX applicator has a large suction cavity on the undersurface of the hand piece with bipolar RF electrodes on each side of that cavity. The hand piece applies suction pressure to the soft tissue of the convex contour irregularity, drawing the skin and subcutaneous tissue up to 1 cm into the cavity. Basic bipolar RF is passed between the two electrodes and through the adipose tissue and skin resulting in tissue heating (Figure 1).

Basic RF heating

The skin and subcutaneous tissue is drawn up to 10 mm into the suction cavity and the RF energy passes between the electrodes, both directly through the fat drawn up into the suction chamber and down into the fat below the electrodes, a distance of 50% of the distance of the two RF electrodes [14]. As the two RF electrodes are 2.5 m apart, the RF heats fat to a depth of 2.2 cm (1.0 cm of suction coupled elevation in addition to the 1.2 cm into the uncoupled fat). The suction cavity and controlled RF bipolar depth distribution allows for a very selective targeting of only the superficial fat and sparing deeper tissues, such as motor nerves or muscle from RF heating of the high voltage, ultra-short pulsing.

As the basic RF heats the tissue, the surface skin and adipose tissue



temperatures both rise. However, because of the deeper thermal RF penetration pathways from the bipolar electrodes and lower resistance of the fat, the adipose tissue temperature rises higher than the skin temperature by 2-4°C [13]. The TiteFX hand piece constantly displays the measured skin surface temperature on the hand piece, as well as on the console. The user is able to set an ‘epidermal cut-off temperature’, which when reached turns off the emitted basic RF energy. With the online temperature monitoring, the treatment professional is able to set the epidermal thermal end point, past which the TiteFX software will turn off the basic RF until the measured skin temperature drops just below the ‘cut-off’ when the RF is turned on again. When the epidermal temperature and the basic RF is turned off, the second RF, or trains of ultra-short (nanosecond), high voltage pulsing (HVP) is then triggered. The ultra-short, high voltage RF pulsing has been shown to produce a cell membrane electroporation and a non-necrotic death through cellular apoptosis in other non-mammalian, mammalian and human cell lines [1-11]. There has been a reported synergy of pre-heating tissue prior with basic RF before the HVP’s in facilitating more effective and efficient irreversible electroporation (IRE) and apoptotic cell death [11,42,43].

The thermal end-point that has been established as optimally efficacious for RF heating is 43-44°C epidermal temperature. Using serial cross sectional thermography on the perfused flaps of abdominoplasty patients as well as thermo-coupled needles on *in-vivo* patients, we were able to determine that the adipose tissue temperatures were 44-45°C at depths up to 2.5-3.0 cm, while the skin temperatures were maintained at a consistent 43-45°C (Figures 2-4).

In vivo studies using a thermal coupler needle confirmed a uniform temperature of 43-44°C from the epidermal surface to a depth of 2.5 cm.

The clinical TiteFX treatment protocol was as follows. The convex distension of localized fat, or cellulite zone to be treated, was marked out with the patient in the standing position. Each treatment zone was divided into two smaller treatment areas, approximately 15×20 cm, or about the size of a human hand (most actual commercial clinical zones, such as outer thighs, love handles, inner thighs, lower abdomen, etc. are the size of two hands, or 30×40 cm). With each “half zone” marked out, the patient is placed in a supine or prone position, depending upon the treatment area. No gel or interface medium is required for the TiteFX.

The treatment parameters are 40-50 watts of energy, 3 second pulse duration, 44°C epidermal thermal “cut off”, and 7-10 High Voltage Ultra-short pulses at the trigger point. The TiteFX applicator is then applied to the skin and the foot pedal switch activated (Figure 5). The skin under the applicator is drawn up into the treatment cavity and basic RF heats the skin and subcutaneous tissue. The tissue is heated for the prescribed 3-second pulse duration and then the applicator is moved to the next adjacent area within the treatment zone, overlapping the previous rectangular treatment area by 10-20%. The treatment technician moves the TiteFX applicator in this fashion around the treatment zone. The epidermal temperature monitor will rapidly rise from starting temperature in the low to mid 30’s to 44°C within approximately 1-2 minutes.

During the TiteFX treatment there is an audible beeping sound when the suction is adequately coupled and the RF is being administered. When the measured epidermal surface temperature gets within 2°C of the set thermal end point (41°C), there is a doubling in the audible beeping and when the end point of 44°C is reached, the audible beep increases to a triple cadence, which alerts the treatment technician that there is no basic RF being administered, as the thermal

endpoint has been reached and the device will emit a train of pre-set 7-10 (depending upon patient comfort) high voltage ultra-short pulses into the same subcutaneous tissue (the actual number of high voltage pulses can be controlled). The treatment technician then simply moves around the treatment zone keeping the area in double beeps (43.9°C), as at this temperature the RF will automatically come back on until the skin is 44°C again, at which time, the RF shuts off. In this fashion, the epidermal skin temperature can be maintained at a uniform and comfortable 44°C, the subcutaneous adipose temperature at 43-45°C and high voltage, ultra-short pulses can be delivered constantly

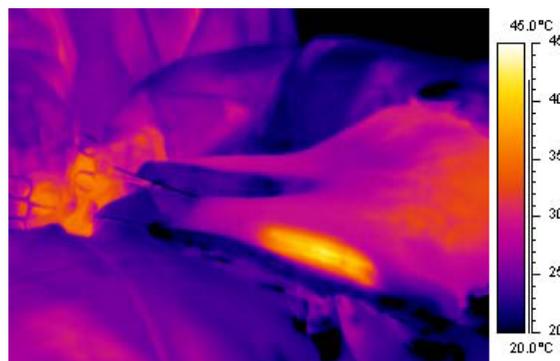


Figure 2: A perfused abdominoplasty flap following 10 minutes of treatment with the TiteFX applicator and thermal camera images showing epidermal temperatures of 43-44°C.



Figure 3: Undersurface of perfused abdominoplasty flap incised and showing adipose temperatures of 43-44°C at levels of 2.5-3.0 cm deep.



Figure 4: *In-vivo* needle Thermo-coupler study measuring the depth of the heating following TiteFX treatment. The thermal profile was found to be a very uniform distribution of 43-44°C epidermal temperature and this was maintained at uniform 43-45° to a depth of 2.5-3.0 cm deep.



Figure 5: TiteFX applicator with Thermal monitoring display on the top surface.

throughout the zone. This modulated temperature control process is called “ACE”, or **Acquire** (*the temperature*)-**Control** (*turn the temperature off-on around the set point*)-**Extend** (*prolong the treatment at the desired temperature to optimize the thermal effect*).

With this automated online ACE temperature monitoring and RF cut-off and HVP protocol, a very homogeneous and uniform temperature profile and heating of the skin and subcutaneous fat can be achieved and more importantly, sustained with good patient comfort. When the epidermal temperature is 43-44°C and the adipose tissue temperature, to a depth of 2.5 cm is also 43-45°C and these conditions are sustained for 10 minutes, there is *in-vitro* and *in-vivo* evidence that an RF mediated thermal heating and destruction of the triglyceride droplets within the cytosol of the adipocyte, which will result in irreversible damage to the adipocyte and over time fat cell necrosis.

It is the exquisite ACE thermal online regulation and cut-off features that allow the adipose tissue heating and ultra-short, HV pulses to be precisely controlled and maintained in this therapeutic adipocyte thermal necrosis range for the necessary 10 minutes. There are few if any “heat spikes” possible, ensuring patient comfort and compliance. When the epidermal endpoint is reached, two things happen:

- (i) Basic RF automatically turns off at 44°C and when the measured epidermal temperature is 43.9°C, the RF comes back on automatically, maintaining a very uniform superficial and hence deep adipose tissue temperature profile. The deep adipose tissue temperature is 2-3°C higher than the skin due to the directional flow of RF into and through the fat from the bipolar electrodes and lower fat impedance.
- (ii) When the pre-set “trigger temperature” endpoint of 44°C is reached, a series of HV pulses is emitted into the adipose tissue to a depth of 2.5 cm. Generally the epidermal thermal endpoint of 44°C is achieved in each zone within one minute and for the next 9 minutes, the technician is continually treating with 3 second pulse durations and then overlapping 10-20% percent

on the next rectangle of adjacent soft tissue throughout the zone, keeping the epidermal temperature at 44°C and emitting trains of 10 High Voltage Pulses with each 3 second treatment cycle.

- (iii) The 15×20 cm zone is treated for 1-2 minutes to achieve critical epidermal end-point temperature with basic RF and then 9 more minutes at this endpoint with intermittent basic RF deployed automatically to maintain the uniform 44°C epidermal and 44°C adipose temperatures and train trains of High Voltage pulses.

High Voltage, Ultra-Short Pulsed RF

The second mechanism that the TiteFX induces a potentially permanent cell injury to the adipocyte cell is through a train of High Voltage, Ultra-Short Pulses (HVP) and fat cell membrane electroporation. When the temperature gets to the monitored target epidermal endpoint that is established by the treatment professional (the “trigger temperature”, generally 44°C), the device will emit a train of up to 10 High Voltage Pulses directly into the adipose tissue until that pulse duration is completed.

The TiteFX allows for very uniform skin heating to a depth of 2.5 cm due to the vacuum suction and electrode placement. Figure 6 shows a thermal image of the skin with a very uniform temperature distribution. The uniformity of the skin temperature means a target epidermal “trigger temperature” of 43-44°C can be reached with minimal patient discomfort.

Irreversible electroporation (IRE) is the process of creating pores and damage in cell membranes under an external electrical field, leading to an influx of calcium ions and an apoptotic death of cells under certain physiological conditions. Preheating of the adipocyte with basic RF current can theoretically allow for a significant reduction in the electroporation threshold [1-11,43,44] and target selectively, as the preheated large adipocytes are then potentially more sensitive to electrical fields [11,43,44]. The TiteFX device uses basic RF and the uniform adipose heating delivered to induce a delayed thermal necrosis of a proportion of the adipose tissue in the treatment field, while the heated adipocytes also become synergistically more sensitized to the HV pulse. In theory, the addition of basic RF heating of the adipocyte cytosolic fat droplet, with the HVP adipocyte apoptosis, the fat cells die over a period of 10 days to 2 weeks and the adipose tissue deflates and loses volume, thereby improving contour [1-11,43,44].

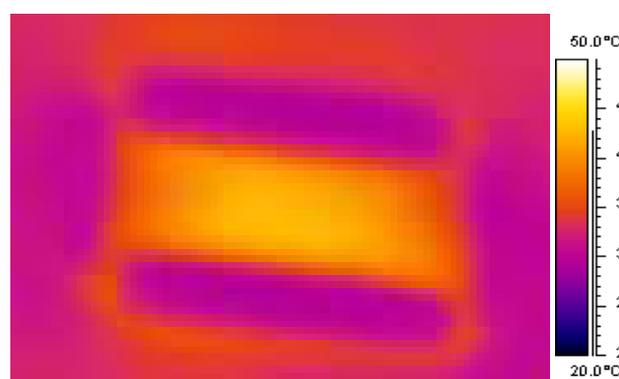


Figure 6: Uniform temperature distribution on treated skin with temperatures of 43-44°C through the treatment zone between the two electrodes.

The third mechanism that basic RF in the TiteFX can result in a body contour improvement and cellulite reduction is skin tightening in the treatment area. The basic RF energy and HVP results in a controlled RF heating of the skin, sustained at 44°C epidermal and a similar and uniform 43-44°C intra-dermal for 10 minutes, which results in heating of the intra-dermal collagen, elastin and extracellular matrix leading to non-ablative, inflammatory injury to the dermis and a wound healing process with enhanced dermal collagen, elastin and ground substance production [13,45]. The production of new dermal type I collagen, neo-elastosis and ground substances takes 5-12 weeks to mature and results in a thicker dermis and a more elastic, or “tighter” dermis. The enhanced thermally mediated skin contraction also contributes to improvement in circumferential reduction, body contour and smoothens the appearance of cellulite by thickening the reticular dermis and reducing the ability for subcutaneous fat to herniated into the deep dermis [46].

Patient Studies

In the current study, 24 female and 1 male patients were enrolled. The age range of the patients was 28 to 62 years old, with an average age of 41.3 yrs. Patient average BMI was 26, but varied from 18 to 30 and all of the patients were interested in focal shape correction, without anticipating any weight reduction. In addition, 14/24 patients also had posterior or anterior grade 2 or 3 thigh cellulite treated.

The patients underwent a 6-treatment TiteFX protocol where they were treated on the abdominal and flank regions once a week over a period of 6 weeks. 14 cellulite patients also had their posterior or anterior thigh cellulite treated. Therapeutic end points were as outlined above, where each zone measuring, 15x20 cm was heated to 44°C epidermal and this was maintained with basic RF and HV pulse RF for 10 minutes. The protocol during the treatment involved moving the TiteFX applicator heating the soft tissue for the three second pulse duration and then moving to the adjacent skin in that zone, overlapping 10-20% with the previous rectangular treatment, rapidly rising the tissue temperature in the 15x20 cm treatment zone to 43-44°C.

Once the trigger temperature of 44°C epidermal was achieved, HVP energy was delivered during the 3-second pulse duration and with 4-6 passes of a train of High Voltage, Ultra-Short Pulses completed in each zone over the 10 minutes. RF power of 40-50 W was used and the treatments were completed in an average 20 minutes per zone. All patients and zones were treated once per week for 6 weeks.

Outcome Measurements

Patients were observed for 3 months to determine the long-term adipose tissue effects and body contour changes. Standard photography and circumferential measurements were taken prior to treatment and at 3 months following the last treatment. Photography was performed using standardized canfield lighting, camera, distances and was always taken during patient exhalation. Patient’s weights and exhalation circumferential measurements were recorded weekly with each visit. 3 patients who were scheduled for future abdominoplasty surgery had Investigational Review Board compliant lower abdominal subcutaneous biopsies performed at 72 hours and 14 days following their initial TiteFX treatment.

All cellulite patients, in addition to photographic assessment, had Vectra 3D cellulite analysis performed on the depths and elevations of the pits and nodules, both pre and post treatment (Figure 7).

Results and Discussion

All patients felt acceptable comfort at epidermal tissue temperatures of 43-44°C and the majority were able to tolerate 44°C. At a skin temperature of 45°C or higher, even with extreme uniformity a significant number of patients experienced discomfort when there was no cooling or topical anesthesia. No adverse side effects were observed at any of these epidermal target temperatures. The train of ultra-short, high voltage pulses created an initial electrical “shock” sensation, but was tolerable in all patients once the patient experience accommodated. Most patients could tolerate 7-10 HV pulses at a time.

Skin erythema, mild edema and a strong heating sensation were typical in the treatment zone following the TiteFX and these lasted up to 6 hours following treatment. The average weight of patients was stable over the treatment course without a significant reduction.

Circumferential reduction was obvious for most patients 3 months following the completion of their 6 treatments. The average circumferential reduction was 3.58 cm with range of 1.5 cm-4.4 cm. There were no non-responders observed in the study.

The visual analogue 10-point patient assessment score for the cellulite improvement showed that over 90% of patients felt there was improvement in the appearance of their cellulite and the average improvement score was 78%. Before and after cellulite photos were evaluated by a blinded physician reviewer and subjected to the modified Nurnberger-Muller 7 stage grading system (Table 1). The average cellulite score improved 2 sub-grades. The average starting pit depth using the Vectra 3D was 4.1 mm (range 0.5 mm-7.3 mm) and the average vectra measured improvement in the pit depth or “smoothness score” was 2.9 mm or 60% (range 1.1 mm-6.3 mm).

The tissue biopsies taken at 72 hours and 14 days after the first treatment revealed no evidence of fat necrosis in any patient. By 14 days, a substantial percent of adipocytes had undergone a histological “shrinkage” of the fat cell (Figure 8). It is not certain, if further death from delayed apoptosis from the HVP would yield further adipose tissue collapse.

Our clinical findings are supported by other *in vitro* and *in vivo* studies of Radiofrequency heating on cultured and live *in situ* adipocytes [45]. Cultured adipocytes show 89% cell viability when subjected to 45°C for 1 minute and 40% when heated at 45°C for 3 minutes. Adipocyte cell viability dropped to 20% when subjected to 50°C for one minute. Other *in vivo* studies have confirmed that maintaining adipose tissue at 44-45°C for 15 minutes at depths of 1.5-2.0 cm resulted in loss

Grade I	Skin is Smooth when standing. Cellulite may be induced by pinching the tissue
Grade II - MILD	Orange Peel or Mattress appearance when standing – MILD
Grade II – MODERATE	Orange Peel or Mattress appearance when standing – MODERATE
Grade II – SEVERE	Orange Peel or Mattress appearance when standing – SEVERE
Grade III – MILD	Cellulite plus raised and depressed areas and nodules when standing – MILD
Grade III – MODERATE	Cellulite plus raised and depressed areas and nodules when standing – MODERATE
Grade III – SEVERE	Cellulite plus raised and depressed areas and nodules when standing – SEVERE

Table 1: The Modified Nurnberger-Muller Cellulite Grading Scale.

of adipocyte viability on biopsy [45]. What is not clear and requires further study is the mechanism of the fat reduction and adipocyte death, as there was no evidence of necrotic cell debris, but rather just fat cell shrinkage, the presumed mechanism was adipocyte apoptosis, again presumably as a result of sustained basic RF heating, the ultra-short, high voltage pulses, or a synergy of both.

The TiteFX and its automated on-line thermal control system allows for a sustained and uniform thermal dose delivery to the subcutaneous fat that is not possible with most other non-thermally regulated systems. Further, the efficacy of the HVP and irreversible electroporation (IRE) is potentially synergized by this sustained deep adipose heating [1-11,43,44].

With the delayed thermal cellular injury and delayed HPV adipocyte apoptosis, the potentially deleterious sudden release of triglycerides by more aggressive disruptive technologies is prevented with TiteFX and the slow apoptotic “inside out” death of the adipocyte by both the RF heating of the triglyceride droplets and the “outside in” ultra-short HVP cell membrane deactivation through IEE. The absence of physically disrupted adipocytes on biopsy at 72 hours and the absence of any lipid spikes on serum blood levels after treatment, support our hypothesis that the adipocytes undergo removal by macrophage phagocytosis without acute cell membrane disruption and significant lipid release.

Our clinical impression is that over time and future studies, there will emerge a time-dose curve, where longer exposure to the predetermined “trigger temperatures” and applying trains of more ultra-short HVP may provide even better results and higher patient satisfaction, but additional clinical studies are underway to refine and optimize the treatment protocols.

Most patients reported a high satisfaction level and were able to detect visible improvement in their body shape. Figures 9-11 show female patients before and 3 months following the TiteFX treatment program. Figure 12 shows a patient three months following anterior thigh cellulite treatment.

The TiteFX cellulite improvements were very consistent. This TiteFX applicator efficacy is likely related to the multiple anti-cellulite effects consisting of:

- (i) Suction causing tensile load on the vertical fibrous septae and a related stress relaxation and lengthening of the vertical fibrous septae reducing the depths of the pits
- (ii) Suction related vasodilatation of superficial subcutaneous vessels and mechanical manipulation leading to improved blood supply, oxygenation and lymphatic drainage
- (iii) Thermal necrosis and/or apoptosis and reduction of the subcutaneous fat, with less fat herniated into the dermis and smoothing the appearance of the “nodules”
- (iv) HVP IRE related apoptosis of the adipocyte population with less intra-dermal herniation and smaller nodules
- (v) RF related thermal thickening of the reticular dermis with more resistance to re-herniation adipose into the sub-dermal fat

Conclusion

The non-invasive treatment of localized deposits of fat and cellulite is one of the fastest growing segments of cosmetic dermatology and aesthetic medicine. While there are a number of body contouring treatment devices on the market, there are few with proven permanent

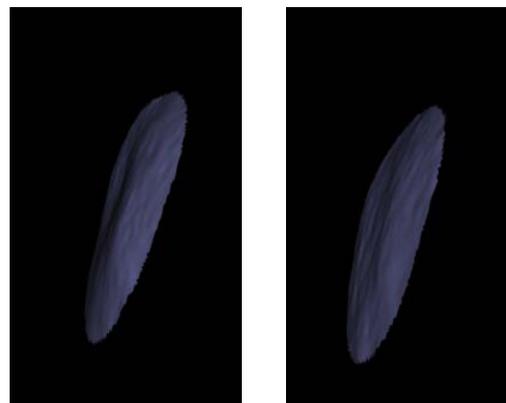


Figure 7: The Vectra 3D topographic photograph showing the decrease in the depth of a cellulite pit following treatment which was calculated through a proprietary Canfield algorithm as being lessened by 3.1 mm or 82%.

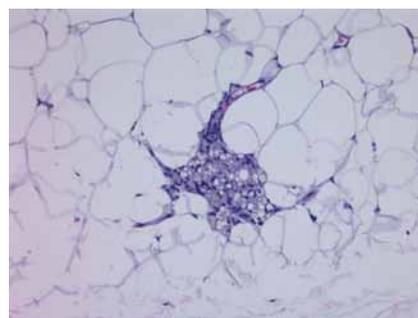


Figure 8: H & E stained superficial adipose tissue showing non-disruptive cell adipocyte cell shrinkage at levels up to 2.5 cm deep.



Figure 9: Female before and 3 months following TiteFX treatment.



Figure 10: Female before and 3 months following treatment with 4.8 cm reduction off her abdomen and hips and a focal bra line reduction.

adipocyte reduction capability available to treat focal fat collections, contour elevations and cellulite, as well as delivering synchronous skin tightening.



Figure 11: Female before and 3 months following treatment with a 6.8 cm reduction off her hip-abdomen circumferential reduction.



Figure 12: Patient with a 81% Canfield vectra measured reduction in the depth the cellulite pits of the lower anterior thigh.

This study on 25 patients provides strong evidence that a combination of suction coupled, selective, controlled RF heating and ultra-short, high voltage RF pulses offers a safe, long term, non-invasive body contouring solution with adipocyte death and therefore long-term contour and cellulite enhancements.

Synergistic controlled RF heating and ultra-short HV pulses appears to result in a consistent circumferential reduction that may be a result of both adipocyte deaths from uniform and sustained heating of the adipose tissue at 43-44°C for 10 minutes, over a series of 6 sessions. In addition, an apoptotic death of adipocytes caused by trains of ultra-short (nanosecond), high voltages pulses, resulting in irreversible electroporation (IRE) effects on the fat cell membrane may also be a major contributor to long-term adipose tissue reduction.

The cellulite improvement was significant and the ability of the device to treat many of the underlying anatomical alterations in cellulitic skin may contribute to its efficacy.

The TiteFX is able to treat both focal fat or cellulite zones, each in 20 minutes, making multiple zone body contouring feasible. There is no disposable costs associated with the use of the device and despite the thermal mechanism and HVP IRE, is very well tolerated by the patient with an apparent high index of safety. The authors believe that the TiteFX technology is effective in delivering a permanent adipocyte apoptotic effect and thus long-term non-invasive fat reduction, cellulite improvement and body contour enhancements. Further studies will help to confirm and position the role of TiteFX in body contouring and cellulite reduction.

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