**Study Title:**
Evaluating the Efficacy of Cellulite Treatment Devices

**Primary Investigator:**
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**Sponsor:**
None

**Introduction/Background:**
Cellulite is present in about 85% of post-pubescent women, which usually affects the thighs and buttocks. The pathogenesis of cellulite includes fibrous septae attachments of the skin extending into the subcutaneous fat connecting to the underlying muscle, a thickened hypodermal fat layer, hypodermal fat lobules herniating into the dermal-hypodermal interface and reduced microcirculation. Ultrasound imaging has been used to view both the skin thickness and the dermis-hypodermis interface. There are currently numerous medical technologies, both minimally invasive and non-invasive, marketed for the treatment of cellulite. However, if one current technology is superior to all the others, this study would be unnecessary. This study is designed to compare both minimally invasive and non-surgical technologies for the treatment of cellulite.

The first modality for the treatment of cellulite was developed in the 1970’s known as Endermologie (LPG Systems, Valence, France). This is a non-invasive motorized mechanical roller that mobilizes subcutaneous fat, temporarily improves microcirculation up to four fold up to 6 hours post-treatment, increases flow velocities in the subcutaneous veins within the adipose tissue, and improves lymphatic drainage by three fold up to 3 hours post-treatment, however, in vivo redistribution of fat was not demonstrated. Additionally, biopsies revealed that Endermologie increases elastin or oxytalan fiber diameter and density, and collagen fibers show a more compact appearance with increased density in the papillary and upper reticular dermis (although the collagen effect is weaker than the elastic fiber effect). Despite these histological changes, there was no change in skin elasticity as measured by a Cutometer. There is no evidence to document if this is a temporary or permanent effect. There was a subjective observed reduction in adipose volume, however, this observation lasted only two weeks after treatment. A porcine study revealed accumulation of dense, longitudinal collagen bands in the mid and deep sub-dermis with distortion and disruption of adipocyte cell membranes. No inflammatory response, skin or muscle injury was observed. The skin and subcutaneous tissue thickness remained constant.

Patients lost a mean weight of 1.35 pounds and a mean body circumference of 0.54 inches undergoing 7 sessions. Another study confirmed these findings with a mean
weight reduction of 0.45 pounds and body circumference of 0.45 inches after undergoing 20 sessions of treatment, although no long term follow-up was performed. Additionally, Endermologie demonstrated softening of superficial skin surface irregularities and cellulite with a 50% improvement by objective blind photographic grading and a 92% patient satisfaction rate. However, objective measures of body contouring were minimal and proportional to weight loss. Thus, Endermologie alone should not be considered an effective body contouring method. Endermologie is approved by the Food and Drug Administration for the temporary improvement in the appearance of cellulite.

Technology using a mechanical roller, infrared light at 700 nm and bipolar radiofrequency was developed and underwent two alterations, known as Velasoom, Velashape I and the newest model, Velashape II (Syneron Medical Inc., Irvine, California). Heat generated by the infrared light and radiofrequency is theorized to increase the dissociation of oxygen from oxyhemoglobin and diffusion into adipose tissue. As with Endermologie, the mechanical massage improves circulation and may stretch the connective tissue bands in the fat layer. Velashape II is approved by the Food and Drug Administration for the temporary improvement in the appearance of cellulite.

A non-focused ultrasound device, VASERshape, manufactured by General Projects (Florence, Italy) and distributed in the United States by Sound Surgical Technologies (Louisville, Colorado) was FDA approved as a non-invasive device for the temporary treatment of cellulite in 2010. The VASERshape system delivers a non-invasive treatment through therapeutic massage and ultrasound diathermy using the combined action of overlapping beams from two ultrasound transducers (1 MHz). This advanced ultrasound handpiece is placed over the skin so that the deep penetrating action of the ultrasound, up to 5 cm, is concentrated onto the affected tissue only, offering a treatment has been shown to be both safe and effective.

The action of ultrasound (ultrasound diathermy) must always be followed with lymphatic drainage. This is performed through the use of the suction/massage handpiece and a special elastomeric membrane, which applies movement to the tissue. This membrane operates in an undulating motion, which gently lifts, folds and compresses the tissue following a sequence of movements specific to each area being treated. This manipulation of the cutaneous and subcutaneous tissues improves lymphatic, arterial and venous circulation. With the same handpiece, the lymph nodes are opened prior to drainage, thus allowing the elimination of potential toxins and lipids in the interstitial spaces caused by the action of the ultrasound.

In the first published series using VASERshape in the US, the multi-center study revealed reductions of 2.1 inches in the abdomen and 1.0 inch in the thighs over 5 treatments performed once per week, and reductions of 0.8 in and 0.5 in, respectively, were achieved after the first treatment. These results were achieved with no adverse events and no patient discomfort.

Due to the less than optimal outcomes for cellulite treatment using any of the non-invasive medical technologies, minimally invasive techniques used for the treatment of cellulite have been brought to market.
The minimally invasive medical technologies to be investigated in this study are the laser and ultrasound energy based devices. The two laser technologies to be investigated are Cellulaze (Cynosure, Westford, Massachusetts) side light fiber at 1440 nm and the Slim Lipo Cellulite device (Palomar Medical Technologies, Burlington, Massachusetts) side light fiber at 924 nm. The non-focused minimally invasive ultrasound device at 36 kHz to be studied is known as VASERsmooth (Sound Surgical Technologies, Louisville, Colorado). Variants of the VASERsmooth device technique include the addition of autologous fat grafting, autologous stem cell grafting and a combination of the fat and stem cell grafting methods.

Minimally invasive laser cellulite treatment was recently evaluated for efficacy, safety and duration of clinical benefit in ten patients using Cellulaze (Cynosure, Westford, Massachusetts), a pulsed laser that delivers energy at 1440 nm using a side firing light fiber. The study revealed that a single treatment observed for at least one year produced a “good” rating by survey. The results showed a patient survey of 3.2 and a physician survey of 3.4 (5 point scale) with a patient satisfaction rate of 93%. Objective measures revealed improved skin elasticity of 29%, by elastometry, and increased skin thickness of 25%, by diagnostic ultrasound. The author concluded the following: 1) uneven dermal-hypodermal interface by melting the hypodermal fat to prevent its expansion into the hypodermis, 2) the connective tissue septae were thermally subcized and 3) the dermal layer due to heat production to increase skin thickness, producing skin tightening and stimulate collagen synthesis.

Palomar Medical Technologies has no published data on their new minimally invasive side firing 924 nm laser cellulite device. Sound Surgical Technologies has no published data on their new minimally invasive VASERsmooth side cutting ultrasound probe device.

The non-invasive technologies to be evaluated in this study include: non-focused ultrasound (1 MHz) or VASERshape (Sound Surgical Technologies, Louisville, Colorado), mechanical massage or Endermologie (LPG Systems, Valence, France) and a combination of infrared light (700 nm), mechanical massage and bipolar radiofrequency or Velashape II (Syneron Medical Inc., Irvine, California).

The goal is to determine the efficacy and the side effects observed with each device in a direct comparison. An additional non-invasive investigation will combine two recognized cellulite treatments, non-focused ultrasound (VASERshape) and mechanical massage (Endermologie) to improve the outcome of each, used separately.

The universal cellulite grading system developed in 1972, the Nurenberger-Muller Grading Scale, was chosen to ensure uniformity in cellulite assessment. There are four grades of cellulite ranging from grade 0-3:

Grade 0: “No cellulite”. There is no visible cellulite while standing. With pinching the skin, there is no appearance of cellulite. This grade of cellulite is rare and very desirable.
**Grade 1:** “Tight cellulite”. There is no cellulite when standing, however, when one pinches the skin of the thigh/buttock or when the thigh/buttock muscles are contracted, one will see the appearance of orange peel or mattress appearance of the skin. The pinch test should be painless and the skin-fold small.

**Grade 2:** “Loose cellulite”. There is visible cellulite while standing, but it is not visible when lying. The cellulite is visible when the subject contracts the buttocks or when the skin is pinched. The skin is generally loose and has a painless skin pinch.

**Grade 3:** There is visible cellulite while standing and in the supine position. Some refer to this as “painful cellulite”, any type of cellulite, which is characterized by significant tenderness during the pinch test.

**Materials & Methods:**
All subjects were healthy women, 18 to 65 years of age who presented with cellulite to the thighs and requested treatment. Exclusion criteria included any medical condition where minimally invasive surgery under oral or mild intravenous sedation was contraindicated, those with documented significant mental illness, nursing home residents, terminally ill, pregnant woman, indigents, prisoners, mentally disabled, institutionalized, hospitalized, and non-readers. Six patients in each different treatment modality or variation of treatment are sought.

The key to the study was a direct comparison on the same patient, where one thigh/buttock with cellulite undergoes one modality and the other side a different modality. The sides for each treatment are determined randomly. The limbs of the study include:

**A. Non-invasive Technologies**
1. Endermologie vs VASERshape followed by Endermologie
2. Vasershape vs VASERshape followed by Endermologie
3. VASERshape vs Velashape II

**Endermologie Technique:**
A subject puts on a body suit. The Endermologie machine is turned on and spontaneously undergoes the calibration process. The treatment personnel validates the parameters on the LCD display to include: time in minutes, which for the thigh is 9 minutes each, and the suction settings can be changed from 1-6, starting at level 3.

The device is then placed on the treatment area; suction is tested to be “on” by the treatment personnel. The rollers are placed on the patient’s skin and the patient is asked if they are experiencing discomfort. If they are tolerating the treatment, suction is steadily increased until the setting where discomfort is elicited. If there is discomfort at the start of treatment the setting is decreased from 3 to 2. The treatment continues at the tolerated
For optimal results when treating different types of cellulite, LPG has specified Pre-Set Program Parameters for each type of cellulite. Those parameters include: Duty cycle %, Frequency (Hz), time for each treatment, and roller speed. The manufacturer recommended settings are as follows:

**Cellulite type 1:** Duty Cycle % 100%, 0.41 Hz, 35 mins, roller speed M  
**Cellulite type 2:** Duty Cycle % 30%, 7.69 Hz, 35 mins, roller speed M  
**Cellulite type 3:** Duty Cycle % 30%, 12.19 Hz, 35 mins, roller speed M

**VASERshape Technique:**
Both lymph nodes and the anterior neck lymphatic duct are opened with manual massage or the massage hand piece for two minutes. Then, using the ultrasound hand piece on modulated ultrasound mode at 60 kHz and at 5 watts/cm², the energy is delivered for 15 minutes. This is followed with continuous mode for 6 minutes at 7 watts/cm². Finally, the massage handpiece is used at 75% suction for a total of 3 minutes at the inguinal area bilaterally.

**Velashape II Technique:**
The machine is turned to the “on” position, calibration spontaneously occurs. The size of the hand piece used (large or small) is determined by having maximum contact with the patient’s skin. Once the sized handpiece is chosen and the booting process is complete, the treating personnel types the settings into the keypad on the hand piece. The standard starting settings of 2,3,2 represent: infrared light (IR), radiofrequency (RF) and suction, respectively. Velashape lotion is then applied to the skin of the treatment area.

Velashape treatment protocol- Patients will undergo once to twice a week treatment for three weeks (total 6 treatments), each treatment duration is for 20—30 minutes, with a goal of each treatment to achieve a skin temperature of a minimum of 40°C with an attempt to achieve up to 45°C. Treatment personnel will apply Velashape treatment oil for conduction during treatment. The handpieces are moved slightly differently. The small hand piece usually needs to be lifted off the patient’s skin and moved to an adjacent site. The large hand piece, in contrast, is pushed onto the adjacent skin area without lifting off the skin surface.

The handpiece is placed on the patient’s skin and striped in one direction with 15% overlap of each strip. The handpieces are moved slightly differently. The small hand
piece usually needs to be lifted off the patient’s skin and moved to an adjacent site. The large hand piece, in contrast, is pushed onto the adjacent skin area without lifting off the skin surface.

Treatment begins by placing the hand piece on the patient as the handpiece is heating up, and with the suction engaged, the treatment personnel counts from 1 to 10. The treatment personnel observes the skin being erythematous and warm to touch.

The settings are altered when the patient experiences discomfort at a level that they can no longer tolerate. If the patient complains of the suction sensation producing too much pain, the suction is decreased from 2 to 1. If the patient states the temperature is too hot, the IR energy setting is decreased from 2 to 1. Then, the new settings are judged to be satisfactory by the treating personnel, again by counting from one to 10. The length of time until the patient complains again of discomfort determines the length of each treatment prior to moving the hand piece to an adjacent area.

Each skin section is then treated for that time period and then moved to an adjacent area. The number of passes is between one, two or three. The target temperature of treatment is 45°C, however, the minimum target temperature is 40°C. The surface skin temperature is measured by a non-contact temperature gun. Treatment is continued until this temperature is reached. Typically, the target temperature is reached in 3-7 minutes.

The manufacturer recommends two treatments per week, attaining tissue temperatures of 40-41°C and treating the patient for about 20-25 minutes per treatment session.

**B. Minimally Invasive Technologies**

1. VASERSmooth vs. Cellulaze
2. VASERSmooth vs. Slim Lipo cellulite device
3. VASERSmooth vs. VASERSmooth adding autologous fat
4. VASERSmooth vs. VASERSmooth adding autologous stem cells
5. VASERSmooth adding fat vs. VASERSmooth adding stem cells & fat
6. VASERSmooth adding fat vs. VASERSmooth adding Platelet Rich Plasma (PRP)

**Laser Technique: (Cellulaze & Palomar Cellulite Device)**

The skin of the thigh and/or buttock affected with cellulite is mapped out in 5 X 5 cm sections with a ruler and sharpie marking pen. The patient is prepped with a prep solution, such as chlorhexidene, and sterile drapes are applied. A wetting solution prepared in a one liter normal saline bag combined with 100 cc of 1% lidocaine, 1-2 mg of 1:1,000 epinephrine and 10 cc of 8.4% sodium bicarbonate. About sixty cc of wetting solution is administered by an infiltration cannula to each of these sections.

A side firing laser fiber is introduced below the skin through a small incision. The amount of laser energy needed to treat each section is determined by the surgeon with
more energy delivered when more fibrous tissue is noted as follows: 800 Joules (J), 1000 J and 1200 J. The target temperature is between 45-47°C.

**VASERsmooth Technique:**
The skin of the thigh and/or buttock affected with cellulite does not need mapping in 5 X 5 cm sections. The skin is marked with a color sharpie marking pen to the areas of indentations and depressions and another color with circles around areas of fat fullness. The patient is prepped with a sterilizing solution, such as chlorhexidine, and sterile drapes are applied. A wetting solution prepared in a one liter normal saline bag combined with 100 cc of 1% lidocaine, 1-2 mg of 1:1,000 epinephrine and 10 cc of 8.4% sodium bicarbonate. Either a tumescent or superwet infiltration technique of wetting solution is administered to the superficial area only using a infiltration cannula to the area of treatment.

Initially, a standard VASER ultrasound probe delivers energy to the entire sub-dermal fat area using the VASER mode of at 60%. This step is performed until loss of tissue resistance is appreciated by the surgeon. Thereafter, the Vasersmooth probe disrupts the fibrous attachments noted by the markings until smooth unabated movement of the probe under the skin at these areas is again appreciated by the surgeon. Areas of excess fat fullness can be suctioned with a small liposuction cannula (3.0 mm). The suction pressure should be at 15-18 mm Hg if fat transfer is to be performed to prevent fat cell injury.

If there are persistent areas of indentations in the area of cellulite, unresolved by cutting sub-dermal fibers by the VASERsmooth ultrasound probe, they may be treated by fat transfer to produce a smooth outcome.

**Three other alternatives for treatment exist:**
1) After completing the standard VASERsmooth cellulite treatment, one can graft a homogeneous thin coat of fat immediately under the sub-dermal fat layer.
2) Alternatively, one can place a thin coat of concentrated stem cells immediately under the subdermal fat layer. Or,
3) A combination of autologous fat mixed with stem cells in a homogeneous thin layer under the subdermal fat layer.

**Fat Transfer Technique:**
Autologous fat is acquired during the cellulite treatment by standard suction assisted fat harvesting into a closed fat collection receptacle. The suction pressure should be between 15-18 in Hg, if fat transfer is to be performed to prevent fat cell injury. Usually a 3.0 mm liposuction cannula is used for harvesting, although 3.7 mm is an alternative.

The fat is separated from the infranatant solution by gravity separation. One may use additional fat processing methods to include:
1) The Adivive fat processing unit, also known as Lipokit (Medi-Khan, Seoul, South Korea)
2) Lipodialysis/PureGraft (Cytori, San Diego, CA)
3) Other fat separation methods currently available, surgeon preference

Stem cells may be acquired by the fat harvesting and fat processing unit Adivive (Medi-Khan, Seoul, South Korea), followed by stem cell concentration and separation using the Maxstem/Celltibator fat processing unit (Medi-Khan, Seoul, South Korea).

Adivive fat processing unit Technique:
Fat harvesting and centrifugation- (this can be found in the product instructions for use (IFU), although it is more clearly noted here.)

i) This step is identical for both the Adivive fat harvesting and purification procedure as well as the first step in the stem cell acquisition and concentration process. Fat is harvested and either transferred or suctioned into the sterile Adivive patented weighted 60 cc syringe (TP-101N).

ii) One loosens the plastic central screw of this syringe three turns using the hexagonal screw driver. This enables the oil to be forced out of the top of the syringe during the centrifugation process.

iii) These TP-101N syringes are placed into the centrifuge using sterile technique with the centrifuge settings between 3500-4000 rpm’s (3500 preferred) for five to eight minutes (eight minutes prefer). (A dummy FPU syringe with 50 cc saline may be needed for counter-balance during the spinning process).

iv) After removing the Adivive fat processing syringe (TP-101N) from the centrifuge, the syringe contains oil that has been ejected through the top of the syringe. This is decanted to a waste container by tipping the syringe on its side.

v) The central screw of the syringe is retightened using the hexagonal screw driver.

vi) The syringe connector (TP-106) is attached to the top of the syringe and then the push stick screw driver (TP-108) is attached.

vii) Each complete turn of the push stick pushes 1 cc of fluid or fat from the TP-101N syringe. The resultant volume of condensed fat, absent infranatant and oil, usually totals
between 25 – 35 cc.

Stem cell concentration and separation using the Maxstem/Celltibator fat processing unit technique:

**Step 1.** Adivive fat harvesting and processing (above)

**Step 2.** Making collagenase enzyme solution for fat dissociation -

i) 5 mg of Liberase MNP-S (Roche, Diagnostics Corp, Indianapolis, IN), a highly purified enzyme blend for tissue dissociation containing highly purified collagenase class 1 and class II from Clostridium histolyticum (GNP grade enzyme) in a powdered form, is mixed with 2 ml of normal saline.

ii) 1 ml of this 2 ml solution is used is drawn up in a 5 cc syringe (the other 1 ml is stored at -21 C in a freezer for the next use). Thus, there is 2.5 mg of Liberase MNP-S collagenase enzyme to be used with about 25 cc’s of the condensed Adivive fat.

According to Roche, the enzyme manufacturer, the lyophilized powdered form of the Liberase MNP-S enzyme can be safely stored for two years and the reconstituted form in normal saline can be store for 6 months.

iii) 1 ml of the collagenase enzyme solution (normal saline along with the Liberase MNP-S) is drawn up and mixed with 24 ml of normal saline, making a total of 25 ml of the collagenase enzyme solution in a 60 cc BD luer lock syringe.

iv) This solution is transferred to a patented 60 cc syringe (TP-102) for use with the Maxstem/Cellibator fat processing unit (FPU). A luer lock adaptor (TP -113) is attached between the luer-lock syringe and the Cellibator syringe (TP-102) to allow for this transfer.
Step 3. Preparation of the enzyme solution with autologous fat cells -

i) Turn on the Maxstem/Celltibator device needed for incubation. It takes about 20-30 minutes for the temperature to reach the target temperature of about 38 C degrees (if the temperature is over 35 C degrees, this is the minimum target temperature). If not reaching 35 degrees C, push stop the button, and then start button again to begin another cycle to increase the temperature to the target temperature of at least 35 degrees C. To confirm the device is turned on, look through window of Celltibator to observe the bottom panel rotating.

ii) 25-30 cc’s (usually 25 cc) of condensed fat acquired from step 1 (Adivive fat harvesting and centrifugation) is transferred to 25 cc’s of the collagenase enzyme solution from step 2, iii.

Note- the Piston plungers are different for each syringe, whereby the Adivive syringe (TP-101N) piston plunger has a tip and the Maxstem/Celltibator syringe (TP-102) piston plunger does Not have a tip).

The direction of the transfer is from the Adivive FPU syringe (TP-101N) to the Maxstem/Celltibator FPU syringe (TP-102) (Not in the opposite direction).
To perform this transfer, the syringe balance weight adaptor (TP-111) is placed on the end of both syringes.

iii) The push stick screw driver (TP-108) is used to push the fat from the TP-101N syringe containing the fat into the TP-102 containing the 25 cc of both the normal saline and the enzyme solution.

iv) The TP-102 syringe now contains about 50 cc of solution (fat, saline and enzyme solution). Remove the syringe weight adaptor (TP-111) and the TP-101N syringe. Place the green cap onto the Celltibator syringe (TP-102). The TP-102 syringe is then shaken 3 to 5 times.

v) This 50 cc admixture in the TP-102 syringe is placed into the sterile rack of the Maxstem/Celltibator container and incubated for 30-35 minutes at 37 or 38 C degrees.

vi) Thereafter, the syringe (TP-102) is taken out of the Maxstem device, and placed in the Adivive centrifuge. It is spun at between 195 and 205 g (RCF- relative centrifugal force) (about 500-600 rpm) for 4 minutes. (Note: when using the TP-102 syringe do NOT open up the piston)

vii) Once removed from the Adivive centrifuge, the screw of the TP-102 syringe is completely removed by the hexagonal driver. The stem cells are at the bottom of the syringe. Do not lean the syringe, keep vertical.
viii) The tubal adapter (TP-109) is screwed into the location of the previously removed screw.

![Tubal Adapter](image1)

ix) The Luer-Luer adaptor (FG-103) is screwed onto the other end of the tubal adaptor (TP-109).

![Luer-Luer Adaptor](image2)

The top of the TP-102 syringe now contains dissociated fat cells, which are to be discarded. The bottom 3-4 cc’s of the TP-102 syringe is the stem cells and stromal fraction.

x) A 60 cc BD luer lock syringe is attached to the luer-luer adaptor (FG-103) and is then pushed down, the top portion of the TP-102 will flow into the BD luer lock 60 cc syringe leaving 3-4 cc of solution.

After removing the TP-102 syringe from the centrifuge do not lean or shake the syringe. Keep the syringe vertically upright and undisturbed leaving about 4 cc of stem cell solution. (typically: top is mild yellow, middle is clear saline with impurities and the bottom is a light pink color corresponding to the stem cells).

![60 cc BD Luer Lock Syringe](image3)

The 60 cc BD luer lock syringe is removed, keeping the adaptor in place.

Step 4: Washing the stem cells

i) The stem cells are isolated by this process, however, the solution needs to be washed of the collagenase enzyme to prevent further cell injury. Washing of this solution occurs 2-3 times with 46 cc of normal saline (resultant 0.00001 concentration of collagenase after one washing). The tubal adaptor (TP-109) as well FG-103 adaptor and BD syringe are removed after adding the 46 cc of normal saline via the luer-luer adaptor (FG-103).
ii) The screw of the piston of the TP-102 syringe is replaced after removing the tubal adaptor (TP-109) and tightened with the hexagonal screw driver.

iii) The syringe is placed back into the Adivive centrifuge at about 200 g for four minutes.

iv) When the cycle is complete, the syringe is removed from the centrifuge. This completes the first washing of the syringe to remove any residual collagenase enzyme. Finally you can find 3-4 cc of the SVF fraction at the bottom of the syringe.

v) Now remove the screw again, and repeat the washing process. Two to three washes are completed. Now there is the TP-102 60 cc syringe with a tight screw containing 50 cc of solution that has been separated.

vi) Now we replace the screw in the TP-102 syringe containing 4 cc of stem cells.

**Step 5: Stem cell addition for fat transfer**

The 2-3 cc of the stem cell volume fraction (SVF) solution is mixed with 25 cc, 50 cc or 100 cc of condensed fat for stem cell fat transfer.

Orthopedic surgery clinically uses this SVF without filtering or, alternatively, alteration with a filter of about 100 microns in size.

i) For fat grafting, one needs 25 cc or 50 cc of the condensed Adivive fat. Connect the syringe weight adaptor (TP-111) is placed on the TP-102 syringe and the 60 cc BD luer lock syringe.

ii) The syringe connector I (TP-106) is placed on the other end of the 60 cc syringe.
iii) Then, the push stick screw driver (TP-108) is attached through the syringe connector (TP-106). Mix back and forth three times to mix stem cells with condensed fat.

iii) Each turn of the push stick moves 1 cc of fat into the fat grafting injection syringe. The condensed fat and stem cell volume fraction (SVF) is transferred into the appropriate sized fat grafting syringes using the Luer lock Adaptor (TP-113) (1 cc for the face and, 5 cc, 10 cc or 20 cc for the body) for fat injection. (Recommend: for the face- mix the stem cells solution with 25 cc or 50 cc; of condensed fat and for the body/breast or buttocks, mix with 50 cc or 100 cc of the condensed fat).

Autologous Fat Collection, The Origins Fat Grafting Assembly and Surgery Usage:
(numerous closed fat collection canisters are available).

The Origins Fat Collection canister is a closed system designed to efficiently and atraumatically collect lipoaspirate during a liposuction procedure.

**Equipment List:**
Canister Lid with Lock Rings: Includes Vacuum Port (1), Patient Ports (2), Auxiliary Port (1), Barbed Port Caps (3), Luer port Cap (1), Large )-Ring (1), and Canister Lid Lock Ring (1). Glass Canister: 125 mm diameter (OD) x 250 mm height.
Canister Base: Includes permanently attached stabilization rods and top rings for stabilization of glass cylinder, Large O-Ring (1), Drain Port (1), Drain Port O-Rings (2), Flow Control Knob (1), and Control Knob O-Rings (2).
Accessories includes 6”silicone Tube (1), Barbed Toomey Syringe Adapter (1), Luer Syringe Caps (3), Toomey Syringe Caps (3), O-Ring Removal Tool (1), Set of O-Ring Replacements (1), and Cleaning Brushes (2)

**Assembly of the Origins Fat Collection Canister System.**
(Inspect system for any cracks, defects or other damage prior to use.
Assemble the Origins Fat Collection Canister System in the sterile field.)
1. There are six O-rings. Seat all six O-rings in the following components: on Canister Base, Canister Lid, Drain Port and Flow Control Knob.
2. Attach Drain Port to Canister Base by rotating clockwise until tight.
3. Attach Flow Control Knob onto Canister Base by aligning the alignment mark on the knob with the alignment dot on the base. Rotate clockwise until tight. When the Flow Control Knob is tight, it is considered to be in the CLOSED position as indicated by rotating adjustment symbol on the Flow Control Knob.
4. Carefully slide the Glass Canister onto the Canister Base. Pushed down on the glass in order to seat around the O-ring, creating a seal.
5. Attach Canister Lid to Glass Container. It should sit tightly with the aid of the O-ring on the Lid. When the Lid is attached, it will allow the Glass Canister to seat tightly on the base when pushing down.

6. Place Lock Ring on top of Canister Lid and rotate to lock. If the lock doesn’t twist over the posts located on the top, press down on the Lid and Canister so the glass pushes completely onto the base, exposing the Posts. Once the Posts are exposed, twist the lock ring to lock the canister system shut.

**Surgical Use of the Origins Fat Collection Canister System.**

a. Connect suction tubing to appropriate cannula/handle and attach other end to a patient port.

b. Connect a second suction tubing to the waste container, which is connected to a vacuum source. Attach the other end of the tubing to the suction source port.

c. Ensure all tubing is securely attached and the canister system is sealed at the base as well as the lid.

d. Set vacuum source to desired vacuum level (15-18 cm H20) and begin the aspiration process.

e. When desired patient results is achieved, or the desired amount of fat is collected, turn off the suction. Vent the canister system by removing or loosening the auxiliary port cap on the lid.

f. DO NOT fill the glass container past the 2000 ml mark.

g. Allow time for gravity to separate the fat from the supernatant.

h. Drain off infranatant into a basin by operating the flow control knob (turning counter-clockwise).

i. When all infranatant is removed, close Flow Control Knob.

j. To transfer from the Origins Fat Collection Canister into a harvesting and re-injection device, attach a luer lock syringe to the drain port. If you are using a Toomey syringe, attach the provided silicone tube to the drain port and attach the barbed Toomey syringe adapter to the other end of the tube.

k. Once the syringe is attached, open flow control knob then pull back syringe to fill. Close flow control knob after syringe is full and detach syringe from the drain port.

l. Transfer collected fat into the appropriate device for further desired use.

Cytori Pure Graft Lipodialysis System: for fat processing after the fat has been harvested using any technique. (this can be found in the product instructions for use (IFU), although it is more clearly noted here.)

**Equipment list:**

Cytori Containing Pure Graft Bag and syringes tops, Cytori Syringe Rack, Cytori Standing Rack with Spatula, 60cc Luer lock syringes (2-4), 60cc Toomey syringes (2-4), Toomey syringe adapters(1 Luer and 1 Toomey), 20cc syringes(body), 1cc syringes (face), Fat Grafting Instrument set, Medium Bowl, Sterile Sodium Chloride.

1. Set up on sterile field, the Cytori Standing Rack and spatula, Cytori Syringe Rack with appropriate size syringes with Toomey and Luer Lock Adapters.
2. Open the Cytori Box using sterile technique, containing the Pure Graft bag and syringe topers. Hang bag on the Cytori Standing Rack hook. Place the waste bag in a dependent position for gravity drainage. Unclip the bag.

3. Remove Fat from the Origins Container by getting a 60cc Toomey syringe, placing Toomey Syringe Adapter and withdrawing the fat. After collecting the fat, the adapter is removed and a Cytori Tip placed on the 60cc syringe. The bag has a port where the Cytori Tip fits into. Place the tip into this port and deposit the fat from the 60cc syringe into the bag. Repeat this procedure a few times and continue depositing fat into the Cytori Bag. Once an appropriate amount of fat is collected (3 to 4 syringes) add 1-2 60cc of sodium chloride through the ad port. Pick up the bag and move bag back and forth for an even mixing of sodium chloride and fat. The fat goes through the purification process allowing impurities to be removed. Continue to add sodium chloride until a good consistent fat color is processed (pale yellow). About 100cc of collected fat yields 50cc of purified fat.

4. Once the fat is processed and the purification is reached, attach a Cytori tip to a 60cc syringe and begin removing the fat though the ad port. Change tip on the syringe to a Luer Lock Adapter and transfer fat to a 60cc Luer Lock or 20cc Luer Lock Syringe. Use fat grafting syringe connectors to transfer fat to the appropriate syringes.

Platelet Rich Plasma (PRP) Acquisition & Processing using RegenLab™ Kit steps: (FDA approved)

1. Withdraw blood from subjects available vein from either an intravenous cannula or via a butterfly.
2. Place the Regen® THT tube into the holder, puncture the tube stopper into the internal needle and fill the tube.
3. The vacuum filing system will collect the required volume of blood (approximately 8 cc/tube).
4. Invert the Regen® THT tube gently several times to mix tube contents.
5. Place the tube into the centrifuge, ensure the tube is counter-balanced with another full tube or a plain (dummy) test tube of equal weight.
6. Set the centrifuge settings for 9 minutes at 3100 rpm.
7. After centrifugation is complete, the cellular components will be clearly separated (plasma straw color fluid superiorly, test tube gel of whitish color in the middle, and red blood cells inferiorly)
8. Approximately 4 cc of concentrated Regen® PRP will be obtained for each Regen® THT tube. Fluid can remain in this tube for up to 6 hours.
9. Place calcium gluconate 10%, 0.5 cc into a 5 cc syringe.
10. Using this syringe, with the 18 gauge needle connected, withdraw the PRP fluid (approximately 4-5 cc). One has about 7-15 minutes to inject these contents before the fluid congeals.
11. Add the contents of this syringe to a syringe containing autologous fat using a fat/PRP ratio of 10:1.
12. Mix the fat and PRP in the syringe 3-5 times.
13. Inject the PRP enhanced autologous fat into the target area.
C. Minimally Invasive and Non-invasive Crossover
Patients who have been treated with either the non-invasive or minimally invasive methods and still have cellulite and who desire additional treatment can decide on the same or different technologies for treatment including the alternative minimally invasive or non-invasive approaches to treatment.

The outcome measures investigated will be:
1. visual appearance documented by video and/or still photography
2. skin thickness measured by non-invasive diagnostic ultrasound measurements (TouchView device)
3. skin elasticity measured by a Cutometer or Elastometer
4. skin tightening measured by surface skin ultraviolet dye tattoos in a triangle pattern changing in size using a black light for visualization
5. measurements of circumference changes using a tape measure at superior middle and inferior points
6. patient and physician observational surveys in a visual analog format regarding discomfort, swelling, bruising and aesthetic outcomes
7. surface skin temperature measurements by non-contact thermometer during the treatment sessions.
8. Some subjects may volunteer for pre and post-treatment 1.5 – 2 mm dermal punch histology biopsies of the treatment area
9. Some subjects may volunteer for a “latex imprint” of the skin pre- and post-treatment

Patients will be observed at one month, three months, 6 and 12 months post-treatment.

Review of the technique of the outcome measures:

1) 35 mm Photography
The key to photography is standard views, anterio-posterior (AP) and lateral, which include the majority of the anatomical area in the photograph against a solid cobalt blue background. The lighting uses side and up down lighting to flash at the time of the camera shutter taking the photograph. This is the same setup for all the before and after photographs.

Video
Using the same background and lighting, a video camera documents the subject at the same height as the treated area (thigh/buttock) in a 360 degree view spinning clock-wise.

2) Skin thickness and fat layer measurements
An external diagnostic ultrasound device, such as the TouchView, distributed by Sound Surgical Technologies, measures the thickness in millimeters (mm) with digital images stored within the system.

3) Elastometer or Cutometer
These medical devices are used to reliably measure skin elasticity. One company manufacturing both devices is Courage+Khazaka (Koln, Germany). The measurement is based on the worldwide acknowledged suction method. Skin is drawn by negative pressure slightly into the aperture of the probe, after some seconds the negative pressure stops and the skin relaxes. The penetration depth of the skin is determined optically during the suction and relaxation. There will be five measurements at each site to optimize accuracy. To ensure the measurement will be at the exact site for each subsequent treatment, an ultraviolet tattoo makes the center of the areas to be assessed.

**Elastometer Procedure:**
Skin is cleansed of makeup and any cream. The device is turned on. The probe is placed flat on the skin with a constant low pressure. When the probe is placed correctly on the skin, the measurement is started automatically. Skin is then sucked within 6 seconds with a negative pressure of 400 mbar. After this 6 second period, the negative pressure stops and the skin returns into its original state. The elasticity is expressed in percent (%) on the display and on the diode chain. This procedure is performed five times to each area for heightened accuracy.

4) Skin Tightening measured by surface skin triangle size changes using ultraviolet dye to produce micro-dots at the three points of the equilateral triangle.

The micro-dots will be applied subcutaneously most commonly using a disposable 3-point round needle and a single use Click Stick Handle (SofTap® Permanent Cosmetics). UV-reactive black light ink (Chameleon Body Art Supply Company) will be used to make the micro-dots. The dots that comprise the triangle will be exactly either 30 mm or 50 mm in length apart. The size of the triangle will be chosen based on the size of the area with indentations caused by the cellulite where the triangle is completely within this area. An accurate measurement between each dot will be recorded immediately after placement for use as a baseline and then at the normal follow-up schedule.

The diameter of each dot is approximately 0.5 mm. With the use of a black light (Wood’s light), the dots appear faintly glowing white in darkness, allowing the surgeon to measure the distance between the dots (and thus skin stretch or retraction) at each follow-up visit.

Tattoo procedures: (three basic methods available)
Method #1- (Supplies are available through any Aesthetician ordering supply company, MEDI Point Blood Lancet-single use)

1. Clean the area with alcohol swab.
2. Position the triangle (5cm) on the of treatment area to identify the three points of the triangle.
3. Pour the ink into the ink well and dip the lancet in the ultraviolet ink.
4. Puncture the skin with lancet a couple of times on the indicated triangle points
5. Use the ultraviolet light to ensure tattoo success.
6. Mark the triangle points with marking pen to proceed to treatment.

Method #2- (Supplies available through Sound Surgical
Comfort System -3R Prong Needle- Single use)

1. Clean the area with alcohol swab.
2. Position the triangle (3 or 5 cm) on the treatment area to identify the three points of the triangle.
3. Assemble the handle and the needle head.
4. Pour the ink into the ink well and dip the needle in the ultraviolet ink.
5. Puncture the skin with the needle a couple of times on the indicated area.
6. Use the ultraviolet light to ensure tattoo success.
7. Mark the triangle points with marking pen to proceed to treatment.

Method #3 – (The tattoo gun can be purchased at www.trilabproducts.com or 877-Tri.Labs, Tri-Lab Product, Inc- Sapphire Pro 110V Gun- Hi Tech Micro pigmentation, Professional tattoo Artist Gun)

1. Clean the area with alcohol swab
2. Position the triangle (5 cm) on the treatment area to identify the three points of the triangle.
3. Assemble the gun with all of the needed attachments.
4. Pour the ink into the ink well and dip the needle 3mm in the ultraviolet ink.
5. Pull out of the ink well and power up for a second. This allows fresh pigment into the tip.
6. Position the gun and fire the application of ink into the designated area.
7. Use the ultra violet light to ensure tattoo success.
8. Mark the triangle points with marking pen to proceed to treatment.

NOTE: The best method of identifying the triangle prior to treatment and after treatment is by using a wood lamp. This method proved to be the most accurate and best method of all. Other ultraviolet lights after the tattoo fades away it creates more of a challenge for an untrained eye.

Supplies needed: Different method of puncture supplies, gloves, cotton balls, alcohol swabs, triangles with circular perforation for consistent results, ultra violet ink and ink wells and ultraviolet light and or a wood lamp.

5) Circumferential measurements (in centimeters) using a tape measure at the superior middle and inferior points of treatment noted before each treatment.

6) Patient and physician observational surveys.
The visual analog scales are given to the patient to complete prior to administering the other scale surveys.
a. Overall satisfaction survey
   i) five point scale (0-worse, 1- poor, 2- moderate, 3- good, 4- excellent)
   ii) 0-100 on a visual analog score (poor to excellent)
b. Cellulite reduction survey
i) five point scale (0- no change, 1- 25% improvement, 2- 50% improvement, 3- 75% improvement, 4- cellulite resolution)
ii) 0-100 on a visual analog score (poor-excellent)
c. Skin texture survey
i) five point scale (0-worse, 1- poor, 2- moderate, 3- good, 4- excellent)
ii) 0-100 on a visual analog score (poor to excellent)

7) A non-contact temperature gun (example; Ryobi, Anderson, SC) at maximum output of <1mW at between 630-670 nm (Class II laser product) is used to determine an accurate surface temperature during the treatment session. The recommended target temperature from the manufacturer of each cellulite treatment modality will be attained during each treatment session. The laser gun documented the final tissue temperature by pointing the gun to the area to be evaluated and reading the LCD screen.

8) Patients volunteering to undergo biopsies had histologic stains performed on the tissue specimens.

**Biopsy technique:**
About 2 cc’s of 1% lidocaine with epinephrine is injected to the proposed biopsy site. A dermal punch (2.5 mm) is placed the flat against the skin, gently pushing downward and twisting in a clockwise-counter-clockwise direction until the skin is detached. If needed, a scissor is used to amputate any residual tissue. The defect is then closed with a suture. The specimen is sent to determine thickness. It is measured with a caliper prior to placing in 10% neutral buffered formalin (NBF) and again at the time of the histologic evaluation. (Note: Placing in formalin fixative will shrink the tissue specimen, however, the same amount for all specimens). The tissue will undergo a stain for elastin fibers (VVG stain) and collagen fibers (Masson trichrome). A qualitative assessment is performed by a board certified dermatopathologist (Dr. Narciss Mobini, MD, Quest Diagnostics) and graded using a 0-10 score comparing before and after treatment biopsies. The dermatopathologist will be blinded to the treatment method used.
References: