Protocol Title: Prospective, single site, controlled comparative study to identify and assess tissue responses after injection with various biostimulatory products

Protocol Version: Version 2.0

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Sponsor: Merz Aesthetics, 6501 Six Forks Road, Raleigh, NC 27615 USA

Principal Investigator(s): Dr Kai Kaye; Dr Gabriela Casabona

Study Site: Ocean Clinic, Av. Ramon y Cajal 7, 29601 Marbella, Spain

ClinicalTrials.gov Identifier: NCT06093815

Study design

Prospective, 2 arm, open label, single-center, injection site-randomized study.

Justification for two arm study

The study has 2 arms and will include *in vivo* analyses. The objective of Arm 1 and Arm 2 of the study is to assess *in vivo* tissue responses to the injection of the products via the use of an ultrasound. In addition, Arm 1 will provide samples for subsequent *ex vivo* histologic analyses and Arm 2 will provide samples for subsequent histologic and *in vitro* assays to assess for prespecified novel biomarkers and gene/protein expression levels (to be defined in a separate protocol). Each subject in Arm 1 will receive all products to allow confidence that differences in the analyses are the results of the product characteristics and not subject characteristics. Up to 5 subjects in Arm 2 will receive only Radiesse® (CaHA) 1:1 dilution and up to 5 subjects will receive only Sculptra® (PLLA) at only the 6 month timepoint. The use of one product per subject will reduce the risk of contamination for the gene/protein expression analyses.

Benefit: Risk assessment

All products used are CE marked. They are considered safe when injected according to their labelling. In addition, the amounts to be injected in this study are well below amounts used in their respective registration studies or in real world clinical use. While there are no direct benefits to the subject, there is also minimal expected risk from these injections. Nevertheless, subjects will be monitored for any adverse events. Although there are no expected direct benefits to the subject, their participation in this study will benefit the advancement of knowledge concerning these products, which will ultimately benefit all future users of these products.

Study synopsis

Sponsor: Merz Aesthetics, 6501 Six Forks Road, Raleigh, NC 27615 USA
Test Device: Radiesse®+ (CaHA), Radiesse® (CaHA) 1:1 dilution, Radiesse® (CaHA) 1:2 dilution, Sculptra® (PLLA), HArmonyCA™ (CaHA+HA), and Ellanse ™(PCL)
Study title: Prospective, single site, controlled comparative study to identify and assess tissue responses after injection with various biostimulatory products.
Version: v0.4
Principle investigator: Dr Kai Kaye and Gabriela Casabona
Study Center Part 1: Ocean Clinic - Av. Ramon y Cajal 7, 29601 Marbella, Spain
Study period: Study participation for each subject will be up to 8 months
Objective: The objective of this study is to identify and assess tissue responses after injection with various biostimulatory products that are already used in the market and have CE approval. The study will assess *in vivo* tissue responses and provide tissue specimens which will be used in a subsequent study, under a separate protocol, which will identify and assess levels of predetermined novel biomarkers and gene and/or protein expression *ex vivo*/*in vitro* after injection with the test devices. All of these assessments will be used to detect differences in tissue responses between different products at various timepoints after injection of the test devices. Assessments under this protocol will include ultrasound of *in vivo* tissue. Assessments using *ex vivo* tissue provided from this study, will include histology of ex vivo tissue and in vitro assays to identify prespecified novel biomarkers and to determine levels of prespecified gene/protein expression and will be defined under subsequent and separate protocol. Safety endpoint: incidence of adverse events.
Methodology This is a prospective, single site, controlled comparative study with 2 arms: **Arm 1** will enroll up to 10 healthy subjects; **Arm 2** will include up to 10 healthy subjects. Screening data is the same for both arms and should be reviewed to determine eligibility. Subjects who are already scheduled for abdominoplasty will be informed of this study and may participate at their will. After informed consent, confidentiality and photographic release forms, participants who meet all inclusion criteria and none of the exclusion criteria should be entered into the study. **ARM 1:** All eligible subjects for **Arm 1** will receive injections of 6 biostimulatory products at 6 months, 3 months, and 2 weeks prior to their abdominoplasty. These products will be injected, per protocol instructions, into the lower abdominal tissue which will be removed at abdominoplasty. Prior to injections being performed each subject will have a 5mm baseline punch biopsy of the lower abdominal tissue. The subjects will receive 14 injections at each injection visit, 2 parallel linear lines per product: (Radiesse®+ (CaHA), Radiesse® (CaHA) 1:1 dilution, Radiesse® (CaHA) 1:2 dilution, Sculptra® (PLLA),

HArmonyCA™ (CaHA+HA), and Ellanse ™(PCL) and 2 saline control injections (42 total injections during the study). The first injection visit will include the baseline biopsy. The lower abdominal flap for each subject will be mapped into 3x3cm squares; each square will represent a saline or product injection site. Individual injection locations will be mapped and documented using an acetate sheet, anchored by anatomical landmarks. Photographs of the lower abdominal flap and measurements may also be used to further document injection sites.

There will be up to four visits by the subjects in **Arm 1**: baseline biopsy and first injection session, second injection session, third injection session and pre op visit. The subject's second injection, third injection and preop visits will include follow up of previous injection(s) prior to the administration of the scheduled injection or preop activities as indicated.

ARM 2: Five eligible subjects in **Arm 2** of the study will receive an injection of 1 biostimulatory product of the 6, Radiesse® (CaHA) 1:1 dilution and five subjects will receive an injection of Sculptra® (PLLA) at 6 months prior to their abdominoplasty. These products will be injected, per protocol instructions, into the lower abdominal tissue which will be removed at abdominoplasty. Prior to injections being performed each subject will have a 5mm baseline punch biopsy of the lower abdominal tissue. The subjects will receive 2 injection at their injection visit of either Radiesse® (CaHA) 1:1 dilution, or Sculptra® (PLLA) and 1 saline control injections (2 total injections during the study). The first injection visit will include the baseline biopsy. The lower abdominal flap for each subject will have a 10x10cm area marked for the product injection. The other areas of the lower abdominal flap will be available for biopsy and saline control. Individual injection locations will be mapped and documented using an acetate sheet, anchored by anatomical landmarks. Photographs of the lower abdominal flap and measurements may also be used to further document injection sites.

There will be up to two visits by the subjects in **Arm 2:** baseline biopsy and first injections, and preop visit. The preop visit will include follow up of the previous injection prior to preop activities.

ARMS 1 and 2: At these follow up visits, subjects will be asked about their previous injection(s) including evaluation for any adverse events related to the injections. They will also undergo non-invasive *in vivo* assessment of the previous injections site(s) to evaluate tissue responses (e.g., changes in dermal thickness, elastosis and microvascular formation). At the time of abdominoplasty, the excised tissue containing the products will be removed in total and submitted to a predetermined lab for histologic and protein and/or gene expression analyses (these activities will be defined in a separate protocol). The subject will be exited from the study at the time of tissue removal and no further study procedures or assessments will be performed.

Assessment will be carried out by Dr Kaye and Dr Casabona the principle investigators.

The number, type and severity of adverse events will be recorded during the duration of the study period.

See table i for outline of study activities.

Note: The removed tissue will be submitted to predetermined lab for histologic and assay analyses. These analyses will be defined under a separate protocol and will only involve *ex vivo* tissue specimens.

Study population: The study will attempt to enroll a broad population of male and/or females with differing ages, Fitzpatrick skin types and ethnicity, where possible. Subjects should already be scheduled for abdominoplasty to be eligible for the study.

Inclusion criteria:

1. Healthy male & females; 22 to 65 years of age and deemed to be a good candidate for abdominoplasty surgery and already scheduled for the procedure.
2. Voluntary participation
3. Ability to comprehend and provide informed consent.
4. Participants agree NOT to use any topical agents containing active ingredients such as retinol, glycolic acid or products that will induce skin peeling or change the appearance of the skin in the treatment area, during the study period.
5. Participants agree NOT to undergo any aesthetic treatments that influence the appearance of the skin (such as Botox, fillers, non-invasive skin tightening, microdermabrasion, laser surfacing, chemical peels etc.) in the treatment area during the study period.
6. Participants agree NOT to undergo any surgical treatments in the treatment area during the study period.
7. Participants agree NOT to take part in another clinical study or undergo other treatments during the study period.

Exclusion criteria:

1. Any previous surgery, including plastic surgery or permanent surgical implant, injectable permanent filler, or fat grafting in the abdominal area.
2. Any previous treatment in the abdominal area including: biostimulatory products, fillers (temporary/long term), toxins, laser, microdermabrasion, micro needling chemical peels, or non-invasive skin tightening treatments (electromagnetic/radio frequency/ultrasound etc.).
3. Planning to receive treatment of any kind in the abdominal area including: biostimulatory products not given as part of the study, fillers (temporary/long term/permanent), toxins, laser, microdermabrasion, micro needling chemical peels, non-invasive skin tightening ((electromagnetic/radio frequency/ultrasound etc.), or fat injections.
4. Acute inflammatory process and/or infection at the injection site (e.g., folliculitis, acne), or history of chronic or recurrent infection or inflammation with the potential to interfere with the investigational results or increase the risk of AEs.
5. Treatment of skin area with dermatosis, e.g., skin tumor, keloid (or extreme keloidal tendency), solar keratosis, warts or birth marks.
6. Eczema, exanthema or open wounds.
7. Previous application of over the counter or prescription, oral or topical, anti-wrinkle or skin enhancer products on the abdominal skin within the past 3 months. Use of continued therapy with some cosmeceuticals (i.e., alpha hydroxyl acids, glycolic acids, skin bleaching agents, retinol, or retinoic acids) in the abdominal area is allowed if the regimen was established and is stable over the last >3 months. In cases of an established regimen of mentioned products at baseline, application should be consistently continued throughout the study.
8. Any contraindication to treatment with biostimulatory products based on the product's IFU.
9. Previous pregnancy or intending to become pregnant during study participation.
10. Known hypersensitivity to biostimulatory products or any of their formulation ingredients.
11. Allergic reaction to topical and local anesthetics.
12. Currently using anticoagulant therapy, e.g., warfarin, heparin, salicylic acid
13. Haemophilia / bleeding disorder.

14. Chemotherapy, radiotherapy or high doses of corticosteroids.
15. Systemic infection (e.g., hepatitis).
16. Uncontrolled diabetes mellitus.
17. Any medical condition that may put the participant at increased risk with exposure to biostimulatory products.
18. Any medication that might modulate the immune response (steroids, biologics etc.)
19. Any other illness or condition that, in the opinion of the investigator, would mean that study participation was not in the best interest of the prospective participant.
20. Is a female of childbearing potential and not using medically effective birth control or is pregnant or lactating. (Note: Urine pregnancy tests will be used in female subjects of childbearing potential.)
21. Any serious disease or disorder (medical or psychiatric) that could, in the opinion of the investigator, interfere with the safe completion of treatment according to this protocol or with study.
22. Injection of local anesthesia into the flap during abdominoplasty surgery.

Criteria for evaluation:

In vivo **Arm 1** and **Arm 2**: Comparison of tissue responses using ultrasound after injection of saline control and products, at follow up visits (e.g., dermal thickness, elastosis, microvascular formation).

Ex vivo tissue analyses, which will be defined in a separate protocol:
Ex vivo **Arm 1**: Comparison of histology at baseline, 2 week, 3 months, and 6 months for all injection specimens (e.g., collagen, elastin, microvascular, etc.)
In vitro **Arm 2**: Assays to identify the presence of predetermined novel biomarkers and gene expression/protein levels at baseline and 6 months for each of the injected products (e.g., proteoglycans, cytokines, T cells, etc.).

Safety endpoints: Incidence and degree of (adverse events) side effects secondary to injections.

Methods of Evaluation.

i. *In vivo* Arms 1 and 2: Ultrasound of injection site tissue analysis by PI
ii. Safety assessment: Adverse event reporting

Ex vivo tissue evaluation:

iii. *Ex vivo* Arm 1: Histologic analyses of all products
iv. *In vitro* Arm 2: Assay analyses to assess gene expression/protein levels of each injected product

Statistical methods:
Descriptive comparison of ultrasound assessments.
Ex Vivo and *in vitro* tissue statistical methods will be defined in separate protocol.

Study protocol
Arm 1 (up to 10 subjects): All subjects will be treated with all products according to their labelling at 6 months 3 months, and 2 weeks prior to abdominoplasty.

Arm 2 (up to 10 subjects): Up to 5 subjects will be treated with Radiesse® (CaHA) 1:1 dilution and up to 5 subjects will be injected with Sculptra®, (PLLA)according to their labelling, at 6 months prior to abdominoplasty.

Procedures	Screening Phone call	Day 0 Visit 1 Arm 1 and 2 180 days prior to Abdominoplasty (+/- 7 days)	Day 1 F/U Email Arm 1 and 2	Day 90 Visit 2 Arm 1 90 days prior to Abdominoplasty (+/- 7 days)	Day 91 F/U Email Arm 1	Day 166 Visit 3 Arm 1 14 days prior to Abdominoplasty (+/- 2 days)	Day 167 F/U Email Arm 1	Day 173 Visit 4 Arm 1 Visit 2 Arm 2 Pre op Visit (+/- 7 days)	Day 180 Visit 5 Arm 1 Visit 3 Arm 2 Day of Abdominoplasty (+/- 7 days)
Screening	X								
Demographics, Med/Surg History, Fitzpatrick	X	X							
Inclusion/Exclusion Criteria	X	X							
Written Informed Consent		X							
Urine Pregnancy Test		X		X		X		X	
Standardized Photography of Abdominal Flap[a]		X		X		X		X	
Baseline Biopsy		X							
Ultrasound Analysis of Injection Sites[a]		X		X		X		X	X

Mapping of Injection Sites		**X**		**X**		**X**			
Injection Procedure		**X**		**X**		**X**		**X**	
Post Injection Email[b]			**X**		**X**		**X**		
Adverse Event Reporting		**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Surgical Procedure									**X**

Table i. Schedule of activities

a. Performed prior to injections
b. Subject will be seen in clinic if warranted

Study narrative

Pre-Study - Screening

1. Volunteers will be screened with the eligibility requirements by telephone prior to Visit 1.
2. Subjects will be given an appointment time for visiting the clinic.

Visit 1 Day 0 Arm 1 and Arm 2 – Baseline and 1st Injection

3. Individuals will be given an informed consent (IC) document & photographic release form, HIPAA form and confidentiality agreement to read. They will have all their study related questions answered by the Principle Investigator or Study Coordinator or Research assistant and if they agree, they will sign the IC/Photography release form, the HIPAA form, and the confidentiality agreement. Subjects will be given one copy of the signed IC/Photography release and of the HIPAA agreement to keep, with the original of each document remaining at the testing facility in the subject's file.
4. Study Coordinator or Research assistant will complete the Consent checklist.
5. Principle Investigator and/or Study Coordinator will review with subject and complete the checklist for all inclusion and exclusion criteria.
6. Subjects will receive a brief examination. Principle Investigator and/or Study Coordinator will review with subject and complete the checklist for medical history and concomitant medications. Principle Investigator and/or Study Coordinator will record demographic data.
7. Females of childbearing age will undergo a urine pregnancy test.
8. Subjects who have completed informed consent and meet the inclusion/exclusion qualifications (to include findings of the physical exam, medical history, pregnancy test and concomitant medications) will be assigned a unique subject number.
9. Principle Investigator, Study Coordinator or Research assistant will record Fitzpatrick skin type.
   Subject will complete and Ethnicity questionnaire

Injection Procedure

1. Subjects will have standard frontal photography of their abdomen recorded by the Study Coordinator or Research assistant. Study Coordinator or Research assistant will sign the photographic record sheet
2. Thoroughly cleanse the skin with a skin disinfectant (Alcoholic clorexidine 2%)
3. For Arm 1 subjects, divide the lower abdominal tissue into 3x3 cm squares, one square per product. For Arm 2 subjects, a single 10 cm area will be marked on the subject's lower abdomen.
4. Mark the injection locations and map with an acetate sheet including skin landmarks.
5. Follow with an application of the disinfecting solution to prepare the area for treatment being careful not to remove the markings
6. After injection of Lidocaine, perform a 5mm punch biopsy as control tissue and prepare for submission to predetermined lab according to local guidelines for transport of a biologic sample.
7. Inject the products and saline control into the lower abdominal tissue with each product in its predetermined area and location.
8. Injections will be performed in accordance with the product's IFU. All injections will be subdermal. For Arm 1 subjects: Two 0.1mL linear injections per product will be placed in one 3x3cm square (total injection volume 0.2

mL per square). One square will receive two 0.1mL saline control injections (0.2 mL). For Arm 2 subjects: either Radiesse® (CaHA) 1:1 dilution or Sculptra® (PLLA) will be injected into a 10x10cm area in the lower abdominal tissue using a fanning injection technique. The other areas of the lower abdominal flap will be available for biopsy and saline control. All injections will use a 22-25gauge 38 mm length cannula. Injection sites may be massaged as needed.

9. Use Ultrasound to ensure the injection is at the appropriate location and depth.

Post Injection Procedure

1. After injections, cleanse the skin with warm water and sterile gauze.
2. Research assistant or study coordinator will discuss post injection instructions.
3. Subjects will be given a date and time to attend the clinic for their next injection.
4. Subjects will be contacted by the research center 24 hours after the injection and will be questioned upon any side effects. Any feedback will be recorded.

Visit 2 Arm 1 Follow up from previous injection and 2nd injection session

1. Subjects will be asked by the Research assistant or Study coordinator if they have experienced any changes in their health or taken new/adjusted current medications since the last visit. The PI will be notified of any changes. Any changes will be evaluated by the PI or SC against the exclusion criteria and recorded.
2. Research assistant or Study Coordinator will query the subject as to any unreported issues the subject has experienced since the last injection. The PI and SC will be informed, the issue assessed as to whether it is an adverse event, and the appropriate forms will be completed.
3. Females of childbearing age will undergo a urine pregnancy test.
4. Standard frontal photograph of the abdomen will be performed.
5. Ultrasound will be performed to assess previous injection sites for product location and any tissue response.
6. Subjects skin will be thoroughly cleansed with a skin disinfectant (Alcoholic clorexidine 2%)
7. Mark the injection locations and map using the subject's acetate sheet including skin landmarks.
8. Follow with an application of the disinfecting solution to prepare the area for treatment and being careful to not to remove the markings.
9. Inject the products and the saline control into the lower abdominal tissue with each product and the control injection in its predetermined area and location. Injections will be performed in accordance with the products IFU. Injection sites may be massaged as needed.
10. Use Ultrasound to ensure the injection is at the appropriate location and depth.
11. After treatment cleanse the skin with warm water and sterile gauze.
12. Research assistant or study coordinator will discuss post injection instructions.
13. Subjects will be given a date and time to attend the clinic for their next injections.
14. Subjects will be contacted by the research center 24 hours after the procedure and will be questioned upon any side effects. Any feedback will be recorded.

Visit 3 Arm 1 Follow up from previous injection and 3rd injection session

1. Subjects will be asked by the Research assistant or Study coordinator if they have experienced any changes in their health or taken new/adjusted current medications since the last visit. The PI will be notified of any changes. Any changes will be evaluated by the PI or SC against the exclusion criteria and recorded. Subjects' diary will be collected.
2. Research assistant or Study Coordinator will query the subject as to any unreported issues the subject has experienced since the last injection. The PI and SC will be informed, the issue assessed as to whether it is an injection site response or adverse event, and the appropriate forms will be completed.
3. Females of childbearing age will undergo a urine pregnancy test.
4. Standard frontal photograph of the abdomen will be performed by the Study Coordinator or Research assistant. Study Coordinator or Research assistant will sign the photographic record sheet.
5. Ultrasound will be performed to assess previous injection sites for product location and any tissue response.
6. Subjects skin will be thoroughly cleansed with a skin disinfectant (Alcoholic clorexidine 2%).
7. Mark the injection locations and map using the subject's acetate sheet including skin landmarks.
8. Follow with an application of the disinfecting solution to prepare the area for treatment and being careful to not to remove the markings.
9. Inject the products and the saline control into the lower abdominal tissue with each product and the control injection in its predetermined area and location. Injections will be performed in accordance with the products IFU. Injection sites may be massaged as needed.
10. Use Ultrasound to ensure the injection is at the appropriate location and depth.
11. After treatment cleanse the skin with warm water and sterile gauze.
12. Subjects will next be seen at the pre op day of their Abdominoplasty procedure
13. Subjects will be contacted by the research center 24 hours after the procedure and will be questioned upon any side effects. Any feedback will be recorded.

Visit 4 Arm 1, Visit 2 Arm 2

1. Subjects will be asked by the Research assistant or Study coordinator if they have experienced any changes in their health or taken new/adjusted current medications since the last visit. The PI will be notified of any changes. Any changes will be evaluated by the PI or SC against the exclusion criteria and recorded.
2. Research assistant or Study Coordinator will query the subject as to any unreported issues the subject has experienced since the last injection. The PI and SC will be informed, the issue assessed as to whether it is an adverse event, and the appropriate forms will be completed.
3. Females of childbearing age will undergo a urine pregnancy test.
4. Standard frontal photograph of the abdomen will be performed by the Study Coordinator or Research assistant. Study Coordinator or Research assistant will sign the photographic record sheet.
5. Ultrasound will be performed to assess previous injection sites for product location and any tissue response.
6. Unless any AE or SAE have been reported and ongoing the subject will be signed out of the study.
7. The excised lower abdominal tissue flap will be collected after surgery and prepared for submission to the predetermined external lab according to local guidelines for transport of a biologic sample.
8. There should be no injection of local anaesthesia into the lower abdominal flap during abdominoplasty surgery.

Methods of Evaluation

Primary efficacy endpoint:

Assessment and comparison via ultrasound of dermal thickening, elastosis, and microvascular formation of 6 biostimulatory products.

Ex vivo tissue analyses endpoints (to be defined in a separate protocol): Assessment and comparison of cellular components of *ex vivo* tissue via histology and *in vitro* levels of prespecified novel biomarkers and levels of gene and/or protein expression via assays after injection with Radiesse® (CaHA) 1:1 dilution or Sculptra® (PLLA).

Patient Reported Outcomes (PRO)

N/A as this study is not intended to treat a deficiency nor deformity but rather provide *in vivo* ultrasound assessments and tissue for *ex vivo* analysis.

Safety endpoints:

Incidence and degree of adverse events (side effects).

Additional methods of evaluation

Digital photography using standard photography of the abdomen (Front facing).
Ultrasound to assess product placement and tissue responses.
Fitzpatrick skin type

Study population: The study should encompass a population of male and/or females who are in good health and have been scheduled to undergo an abdominoplasty procedure with 6 months of entering the study.

Inclusion criteria:

- Healthy male & females; 22 to 65 years of age and deemed to be a good candidate for abdominoplasty surgery and already scheduled for the procedure.
- Ability to comprehend and voluntarily provide informed consent.
- Participants agree NOT to use any topical agents containing active ingredients such as retinol, glycolic acid or products that will induce skin peeling or change the appearance of the skin in the treatment area, during the study period.
- Participants agree NOT to undergo any aesthetic treatments that influence the appearance of the skin (such as Botox, fillers, microdermabrasion, laser surfacing, chemical peels etc.) in the treatment area, during the study period.
- Participants agree NOT to take part in another clinical study or undergo other treatments during the study period.
- Participants agree NOT to undergo any surgical treatments in the treatment area during the study period.

Exclusion criteria:

- Haemophilia / bleeding disorder
- Uncontrolled diabetes mellitus
- Treatment of skin area with dermatosis, e.g. skin tumor, keloid (or extreme keloidal tendency), solar keratosis, warts or birth marks
- Anticoagulant therapy, e.g. warfarin, heparin, salicylic acid
- Chemotherapy, radiotherapy or high doses of corticosteroids
- Systemic infection (e.g. hepatitis) or acute skin infection (e.g. herpes)
- Any form of active acne
- Allergic reaction to topical and local anesthetics
- Lactation
- Previous pregnancy, current pregnancy, or intending to become pregnant during study participation.
- Eczema, exanthema or open wounds
- Any previous surgery, including plastic surgery or permanent surgical implant, injectable permanent filler, or fat grafting in the abdominal area.
- Is a female of childbearing potential and not using medically effective birth control or is pregnant or lactating. (Note: Urine pregnancy tests will be used in female subjects of childbearing potential.)
- Any previous or planned treatment in the abdominal area including: biostimulatory products, fillers (temporary/long term), toxins, laser, microdermabrasion, micro needling chemical peels, or non-invasive skin tightening treatments (electromagnetic/radio frequency/ultrasound etc.), or fat injections.

- Acute inflammatory process and/or infection at the injection site (e.g., folliculitis, acne), or history of chronic or recurrent infection or inflammation with the potential to interfere with the investigational results or increase the risk of AEs.
- Previous application of over the counter or prescription, oral or topical, anti-wrinkle or skin enhancer products on the abdominal skin within the past 3 months. Use of continued therapy with some cosmeceuticals (i.e., alpha hydroxyl acids, glycolic acids, skin bleaching agents, retinol, or retinoic acids) in the abdominal area is allowed if the regimen was established and is stable over the last >3 months. In cases of an established regimen of mentioned products at baseline, application should be consistently continued throughout the study.
- Any contraindication to treatment with biostimulatory products based on the product's IFU.
- Known hypersensitivity to biostimulatory products or any of their formulation ingredients.
- Any medical condition that may put the participant at increased risk with exposure to biostimulatory products.
- Any medication that might modulate the immune response (steroids, biologics etc.)
- Any other illness or condition that, in the opinion of the investigator, would mean that study participation was not in the best interest of the prospective participant.
- Any serious disease or disorder (medical or psychiatric) that could, in the opinion of the investigator, interfere with the safe completion of treatment according to this protocol or with study.
- Injection of local anaesthesia into the flap during abdominoplasty surgery.

Concurrent medication

All subjects should be maintained on the same medication throughout the entire study period, as medically feasible, with no introduction of new chronic therapies. Changes in concomitant medication will be recorded at each visit.

Prohibited Medication and Treatments.

Those medications listed in exclusion criteria. Any topical agents containing active ingredients such as retinol, glycolic acid should not be used in the treatment area, during the study period.

Injection procedure for the test products

1. Subjects will have standard frontal photography of their abdomen recorded by the Study Coordinator or Research assistant. Study Coordinator or Research assistant will sign the photographic record sheet
2. Thoroughly cleanse the skin with a skin disinfectant (Alcoholic chlorhexidine 2%)
3. Prior to the first series of injections and after injection with Lidocaine, a 5mm biopsy will be taken as a baseline sample and prepared for submission to a predetermined lab according to local guidelines for transport of a biologic sample.
4. Injections will be performed in accordance with the product's IFU. All injections will be subdermal. For Arm 1 subjects: Two 0.1mL linear injections per product will be placed in one 3x3cm square (total injection volume 0.2 mL per square). One square will receive two 0.1mL saline control injections (0.2 mL). For Arm 2 subjects: either Radiesse® (CaHA) 1:1 dilution or Sculptra® (PLLA) will be injected into a 10x10cm area in the lower abdominal tissue using a fanning injection technique. The other areas of the lower abdominal flap will be

available for biopsy and saline control. All injections will use a 22-25gauge 38 mm length cannula. Injection sites may be massaged as needed.

5. Mark the injection locations and map with an acetate sheet including skin landmarks.
6. Follow with an application of the disinfecting solution to prepare the area for treatment and being careful to not remove the markings.
7. Inject the products into the lower abdominal tissue with each product in its predetermined area and location. Injections will be performed in accordance with the products IFU. Injection sites may be massaged as needed.
8. Use Ultrasound to ensure the injection is at the appropriate location and depth.

Post Injection Procedure

5. After injections, cleanse the skin with warm water and sterile gauze.
6. Research assistant or study coordinator will discuss post injection instructions.
7. Subjects will be given a date and time to attend the clinic for their next injection.
8. Subjects will be contacted by the research center 24 hours after the injection and will be questioned upon any side effects. Any feedback will be recorded.

Adverse event procedure

Definition of an adverse event

An adverse event (AE) is any untoward medical occurrence in a clinical investigation of a product that does not necessarily have a causal relationship with the treatment. An AE is therefore any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the administration of an investigational product, whether or not related to that investigational product. An unexpected AE is one of a type not identified in nature, severity, or frequency in the current Investigator's Brochure or of greater severity or frequency than expected based on the information in the Investigator's Brochure**.**

Evaluation of Adverse Events

At each evaluation/assessment, the PI will determine whether any adverse events have occurred. The subject will be questioned in a general way, and no specific symptoms will be suggested. If any adverse events have occurred, they will be recorded on the AE form. This is in preference to the listing of individual signs and symptoms. Adverse event reporting will begin from day 1. Reporting of new adverse events will continue to be followed up to the final follow-up visit.

The intensity of all AEs will be graded as follows:

1 Mild: -Awareness of symptoms but easily tolerated
2 Moderate: -Discomfort enough to cause some interference with daily activities
3 Severe: -Incapacitating, with inability to work or do usual activity

The PI will make a judgment regarding whether the AE was considered related to the study device according to the definitions below:

None	-	The adverse event is not related to the study procedure/conditions, i.e. it has no association with the study treatment.
Unlikely	-	the adverse event onset and the study therapy commencement is such that the study therapy is not likely to have any reasonable association with the adverse event
Possible	-	the adverse event could have been produced by the study therapy.
Probable	-	the adverse event follows a reasonable temporal sequence from the time of study therapy commencement, abates upon discontinuation of the study therapy.
Highly Probable	-	the adverse event follows a reasonable temporal sequence from the time of study therapy commencement, abates upon discontinuation of the therapy and reappears when the study therapy is re-introduced

Follow-up of Adverse Events

If any adverse events are on-going when a subject completes the study, the surgeon will be consulted as to whether the tissue containing the adverse event was entirely removed at the time of surgery and therefore is no longer a concern. If the tissue containing the adverse event is remaining, the subject will be evaluated in 2 weeks. If the subject is withdrawn from the study with an ongoing adverse event, the subject will be re-evaluated within 2 weeks. If any adverse event has not resolved at 2 weeks after surgery or 2 weeks after subject withdrawal from the study, additional follow-up will be performed as appropriate. Every effort should be made by the PI or delegate to contact the subject until the adverse event has been resolved. This should be documented in the CRF.

Reporting Adverse Events

In the case of a Serious Adverse Event (SAE) the PI must be within 24-48 hours of the investigator becoming aware of the event:
Complete an Adverse Event Form.
SEND (by email within 24 – 48 hours of becoming aware of event) the signed and dated AE form to the study sponsor and the EC.
The Investigator will be requested to supply as much detailed information regarding the event that is available at the time of the initial contact on the study-specific AE form. The investigator is also required to submit follow-up reports until the AE has resolved.

Discontinuation and replacement of subjects

Early Discontinuation of treatment

i. A subject may be discontinued from study treatment at any time if the subject, the PI, or the Sponsor feels that it is not in the subject's best interest to continue. The following is a list of possible reasons for study treatment discontinuation:
    a. Subject withdrawal of consent (or assent)
    b. Subject is not compliant with study procedures
    c. Adverse event that in the opinion of the PI would be in the best interest of the subject to discontinue study treatment
    d. Protocol violation requiring discontinuation of study treatment

e. Lost to follow-up
f. Sponsor request for early termination of study
g. Positive pregnancy test (females)

If a subject is withdrawn from treatment due to an adverse event, the subject will be followed and treated by the PI until the abnormal parameter or symptom has resolved or stabilized.

All subjects who discontinue the study treatment should come in for an early discontinuation visit as soon as possible and then should be encouraged to complete all remaining scheduled assessments.

All subjects are free to withdraw from participation at any time, for any reason, specified or unspecified, and without prejudice.

The PI will provide a reason for subject withdrawals. The reason for the subject's withdrawal from the study will be specified in the subject's source documents. Where a subject does not attend a study visit(s) and does not contact the research center the research center will make reasonable attempts to contact the subject.

Three telephone calls will be made to the subject (3-7 days apart) using the contact details given by the subject. If the research staff are unable to speak to the subject directly a voicemail will be left asking the subject to contact the research center. If an email address is on file, then in addition the subject will be contacted by email on 2 separate occasions 7 days apart.

Withdrawal of Subjects from the Study

A subject may be withdrawn from the study at any time if the subject, the investigator, or the sponsor feels that it is not in the subject's best interest to continue.

All subjects are free to withdraw from participation at any time, for any reason, specified or unspecified, and without prejudice.

Reasonable attempts will be made by the investigator to provide a reason for subject withdrawals. The reason for the subject's withdrawal from the study will be specified in the subject's source documents. Subjects who discontinue study treatment early (i.e., they withdraw prior to Visit 3) should have an early discontinuation visit and should be encouraged to come in for their next scheduled visit (procedures to be followed would include those for their next scheduled visit, save the treatment procedure).

An off-study form will be completed**.**

Protocol Violations and Deviations

Protocol Deviation

The study will be conducted as described in the protocol. However, the PI may deviate from the protocol to eliminate an immediate hazard to subjects without prior IRB approval. The PI or designee should document and explain any deviation from the approved protocol due to an emergency, accident, or error. As soon as possible after such an occurrence, the PI should submit the implemented deviation or change,

the reasons for it, and any proposed protocol amendment(s) to the IRB, to the Sponsor or designee, and to the regulatory authorities, if required.

A deviation from the protocol is an unintended and/or unanticipated departure from the procedures and/or processes approved by the sponsor and the IRB and agreed to by the PI. Deviations usually impact individual subjects or a small group of subjects and do not involve inclusion/exclusion or primary endpoint criteria.

Protocol violation

A protocol violation occurs when the subject, PI, or sponsor fails to adhere to significant protocol requirements affecting the inclusion, exclusion, subject safety, and primary endpoint criteria. Protocol violations for this study include, but are not limited to, the following:

i. Failure to meet inclusion/exclusion criteria

ii. Use of a prohibited concomitant medication

iii. Include other specific examples as appropriate for the study (non-compliance with study drug regimen, etc.)

iv. Failure to comply with Good Clinical Practice (GCP) guidelines will also result in a protocol violation. The Sponsor will determine if a protocol violation results in withdrawal of a subject.

When a protocol violation occurs, it will be discussed with the investigator and a Protocol Violation Form detailing the violation will be generated. This form will be signed by a Sponsor representative and the Investigator. A copy of the form will be filed in the site's regulatory binder and in the Sponsor's files**.**

Statistical Methods and Considerations

Data Sets Analyzed
All eligible patients who are enrolled into the study and receive at least one series of injections will be included in the safety analysis.

Analysis of endpoints
Primary endpoint analyses will be compared per patient and per product and after pooling for each product and timepoint. Trends will be described.

Safety information will be presented using descriptive analyses.

Sample size
Up to 16 subjects scheduled for abdominoplasty will be enrolled.

Data Collection, Retention and Monitoring

The study will be conducted, and study documentation (electronic or paper) will be completed and maintained in accordance with Good Clinical Practices, 21 CFR Part 11 and instructions provided by the

Sponsor. All requested protocol data will be captured onto the relevant source document within the Subject file.
Each page will be identified with the Study identification number with Subject ID and date. Upon completion, the designated member of staff will sign and date the page.
Research staff will transfer study data from the Source document to the corresponding electronic case report form (eCRF).
The study monitors will review the data and study documentation (if applicable) as outlined in the Study Monitoring Plan.
Subjects will not be identified by name in any study documents to be collected by the Sponsor (or designee) but will be identified by a subject number and initials. The Investigator is responsible for all information collected on subjects enrolled in this study. All data collected during this study must be reviewed and verified for completeness and accuracy by the Investigator. A copy of the Source documents and eCRF will remain at the Investigator's site at the completion of the study.
If a correction is required for an eCRF, the time and date stamps track the person entering or updating eCRF data and creates an electronic audit trail. For paper Source documents, if a correction is made on a document, the study staff member will line through the incorrect data, write in the correct data and initial and date the change.
Paper Source documents will be stored within a locked secure storage file that is contained within a locked room. All efiles used for study purposes are double password protected.
The Investigator must make study data accessible to the monitor, other authorized representatives of the Sponsor (or designee), IRB/IEC, and Regulatory Agency inspectors upon request.
A file for each subject must be maintained that includes the signed Informed consent and copies of all source documentation related to that subject. The Investigator must ensure the reliability and availability of source documents from which the information on the CRF was derived.
All study documents (patient files, signed informed consent forms, copies of CRFs, Study File Notebook, etc.) must be kept secured for a period of 3 years following the end of the study. There may be other circumstances for which the Sponsor is required to maintain study records and, therefore, the Sponsor should be contacted prior to removing study records for any reason.
Electronic files transferred between the research center and sponsor must be password protected.

Monitoring
Monitoring visits will be conducted by a representative of the Sponsor according to the U.S. CFR Title 21 Parts 50, 56, and 312 and ICH Guidelines for GCP (E6).
The monitoring plan has been designed using FDA guidance document (Oversight of Clinical Investigations – A Risk Based Approach to Monitoring.
By signing this protocol, the Investigator grants permission to the Sponsor (or designee), and appropriate regulatory authorities to conduct on-site monitoring and/or auditing of all appropriate study documentation.

Subject Confidentiality
To maintain subject confidentiality, only subject number and letters will identify all study subjects on CRFs and other documentation submitted to the Sponsor.

Administrative, Ethical, Regulatory Considerations

The study will be conducted according to the Declaration of Helsinki, Protection of Human Volunteers (21 CFR 50), Institutional Review Boards (21 CFR 56), and Obligations of Clinical Investigators (21 CFR 312) and in accordance with Good Clinical Practice.

To maintain confidentiality, all evaluation forms, reports and other records will be identified by a coded number and letters only. All study records will be kept in a locked file cabinet and code sheets linking a patient's name to a patient's identification number will be stored separately in another locked file cabinet. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by regulatory bodies. The Investigator must also comply with all applicable privacy regulations (e.g., Health Insurance Portability and Accountability Act of 1996, EU Data Protection Directive 95/46/EC).

Protocol Amendments

Any amendment to the protocol will be written by the Sponsor. Protocol amendments cannot be implemented without prior written IRB/IEC approval except as necessary to eliminate immediate safety hazards to patients. A protocol amendment intended to eliminate an apparent immediate hazard to patients may be implemented immediately, provided the IRB or EC is notified within five working days.

Independent Ethics Committees (EC) / Institutional Review Boards (IRB)

The protocol and consent form will be reviewed and approved by suitable Independent EC of the participating study center prior to study initiation. Serious adverse experiences regardless of causality will be reported to the EC in accordance with the standard operating procedures and policies of the EC, and the Investigator will keep the EC informed as to the progress of the study. The Investigator will obtain assurance of EC compliance with regulations.

Any documents that the EC may need to fulfill its responsibilities (such as protocol, protocol amendments, Investigator's Brochure, consent forms, information concerning patient recruitment, payment or compensation procedures, or other pertinent information) will be submitted to the EC. The ECs written unconditional approval of the study protocol and the informed consent form will be in the possession of the Investigator before the study is initiated. The ECs unconditional approval statement will be transmitted by the Investigator to the Sponsor or designee prior to the shipment of study supplies to the site. This approval must refer to the study by exact protocol title and number and should identify the documents reviewed and the date of review.

Protocol and/or informed consent modifications or changes may not be initiated without prior written EC approval except when necessary to eliminate immediate hazards to the patients or when the change(s) involves only logistical or administrative aspects of the study. Such modifications will be submitted to the EC and written verification that the modification was submitted and subsequently approved should be obtained.

The EC must be informed of revisions to other documents originally submitted for review; serious and/or unexpected adverse experiences occurring during the study in accordance with the standard operating procedures and policies of the EC; new information that may affect adversely the safety of the patients of the conduct of the study; an annual update and/or request for re-approval; and when the study has been completed.

Statistical Analysis

Data from subjects who complete the study will be included in all statistical analyses of the primary endpoints.

Skin thickness will be assessed by averaging three measurements per ultrasound image. Results will be reported descriptively as mean absolute values per treatment and time point.

Elastosis, indirectly quantified by the subepidermal low-echogenic band (SLEB) within the dermis, will be reported as an ordinal categorical variable classified as “absent”, “focal”, or “continuous”.

Microcirculation, evaluated by Power Doppler ultrasonography, will be reported as an ordinal categorical variable classified as “diminished”, “normal”, or “augmented”.

Statistical evaluation of comparisons between study treatments and timepoints will be conducted for skin thickness and elastosis according to the **Statistical Analysis Plan**. For study arm 1, analyses will include differences between months 3 and 6 compared with the corresponding baseline. For study arm 2, analyses are limited to a comparison between baseline and month 6.

Statistical Analysis Plan

Evaluation	**Change from Baseline**	**Comparisons between study treatments and timepoints**	**Notes/Interpretation**
Skin thickness	Percentage change in skin thickness at 3 and 6 months will be calculated as: %Δ Skin thickness = (Treatment value - Baseline value)/Baseline value x 100	Percentage change in skin thickness will be analysed using a two-way ANOVA (factors: treatment and time), followed by Tukey’s post hoc multiple comparisons test	A positive and significant percent change indicates an improvement in skin thickness.
Elastosis	Changes in elastosis categories between baseline and follow-up time points (3 or 6 months) will be analysed using the Stuart–Maxwell test for marginal homogeneity. χ^2, degrees of freedom (df), and p-value will be reported.	-	A statistically significant p-value indicates a change in the overall distribution of elastosis categories over time.
Microcirculation	-	-	No formal statistical tests are planned for this parameter.

Statistical analyses of ex vivo tissue endpoints for study arms 1 and 2 are defined in a separate protocol.