Assessment and Augmentation of Lip Appearance in Specific Study Populations

NCT04839692

December 29, 2021

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VERSION DATE
December 29,2021

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I. OBJECTIVES

The objectives of this study are:

- To determine the effects of aging on the lips and perioral area by comparing quantitative changes in lip; and
- To validate the role of optical coherence tomography (OCT) in investigating lip microcirculation; and
- To evaluate any volume, color and texture change of the lips pre and post treatment
- To investigate pain levels from lip filler treatment

To achieve the objectives, the study aims to obtain data from two specific study populations, including:

1) Aged females, otherwise healthy (≥ 60 years);
2) Young females, otherwise healthy (21-30 years)

II. BACKGROUND

Skin aging is a complex biological process mainly influenced by two independent mechanisms, intrinsic and extrinsic skin aging [1]. Intrinsic skin aging is determined primarily by inherent genetic factors, hormonal status, cellular metabolism and function [2]. It represents degenerative aging processes seen in other organ systems as a consequence of the passage of time. Extrinsic skin aging results from various external influences including solar UV radiation, health and lifestyle factors, such as tobacco smoking, environmental pollution, and other systemic conditions, such as diabetes and renal insufficiency [3-5].

In skin with chronic exposure to UV radiation, termed photoaging, alterations in skin structure and function depend primarily on the degree of sun exposure and baseline skin pigment. UV radiation induces collagen breakdown. Diminution of collagen framework that supports vascular plexus within the skin leads to broken vessels [8]. The Langerhans cell population representing immune system of the skin is found to be reduced after chronic exposure to UV radiation contributing to impaired immune response to antigen and skin cancer cells [6,7].

These age-related changes caused by chronic sun exposure have not been well-documented in the lips [10]. The majority of literature on the aging lip has focused on volume loss. Hyaluronic-acid based fillers have been used primarily for volume augmentation, but their effect on lip color and texture has seldom been defined [9,12].

We propose to study aging of the cutaneous changes of human skin anatomy in the lips and perioral area to better understand and quantify the aging process in this, including the effect of age on lip redness.

We also aim to test the effects of a common lip augmentation procedure, hyaluronic-acid based fillers, on the appearance of aging lips, in addition to the tolerability of this procedure. We hypothesize that dermal lip fillers may decrease the redness of the lips.

Besides, we want to evaluate oxytocin level changes and relate it to subjects' satisfaction with procedure.

The study will take place at MGH's Clinical Unit for Research Trials & Outcomes in Skin (CURTIS) at 50 Staniford Street, Suite 240 Boston, MA 02114 or Translational Clinical Research Center (TCRC) on the 12th floor of the White Building.

III. Specific AIMS

To:

- Compare the changes in skin anatomy due to intrinsic aging
- Correlate clinical assessment with OCT imaging;
- Compare changes in lip color, texture and volume before and after filler with non-invasive imaging, including digital photography, OCT and three-dimensional imaging
- To assess the tolerability of lip filler by evaluating pain level from lip filler treatment at initial insertion and overall procedural pain
- To evaluate how oxytocin levels change during and after an aesthetic procedure.
- To assess lip color change after the procedure.
- To evaluate patient satisfaction related to the procedure.

IV. SUBJECT SELECTION

Subjects will be screened to determine if they meet all the eligibility criteria specified below. We will recruit and screen up to 50 subjects with the goal of 40 subjects enroll and complete the study (20 per study arm).

a. Inclusion Criteria

1. Subjects must be able and willing to provide written informed consent and comply with the requirements of the study protocol;
2. In good general health, based on answers provided during the screening visit;
3. Subjects must be able to read and understand English;
4. Female subjects;
5. Aged 21-30 years and Post menopausal women;
6. Skin Types (Fitzpatrick Skin Phototypes I-VI);

* Smoking history is self-report.

7. Have very thin to moderately thick lips (Lip Fullness Grading Scale [LFGS] scores 0–2).

b. Exclusion Criteria

1. Participation in another investigational drug or device clinical trial in the past 30 days;
2. Are pregnant or lactating;
3. Intake of hormone replacement therapy (HRT) in the past 6 months;
4. Have a history of drug or alcohol abuse or have reported habitual alcohol intake greater than 2 standard drinks per day [e.g., 2 beers, 2 glasses of wine, or 2 mixed drinks];
5. Presence of eczema, psoriasis, or any other skin disease on the lips or perioral skin;
6. Have birth marks, tattoos, scars, or any other disfiguration of the skin in the skin area of interest;
7. Use of any anti-aging skin care products containing retinoic acid, retinol, or other retinoids (e.g. tazarotene, adapalene) or estrogen on the skin area of interest in the past 6 months;
8. Use of any prescription topical medication, such as corticosteroids or hydroquinone on the skin area of interest in the past 6 months;
9. Have a history of photodynamic therapy treatment or any skin rejuvenation procedure on the skin area of interest;
10. History of blood-clotting abnormality;
11. History of keloid formation or hypertrophic scarring;
12. Clinically significant abnormal findings or conditions which might, in the opinion of the Investigator, interfere with study evaluations or pose a risk to subject safety during the study;
13. Exhibits any clinical conditions or takes any medication which in the opinion of the investigator may interfere with the study or pose a risk to subject safety during the study;
14. Is not able to follow study protocol;
15. Have permanent lip implant, and lip enhancement or laser therapy performed within the preceding 12 months;
16. Known history of allergy or sensitivity to glycerol, Tegaderm, lidocaine, hyaluronic acid dermal fillers or materials with gram-positive bacterial proteins.

c. Source and Recruitment of Subjects

The study will be posted on the Partner's clinical research web page (Rally.partners.org) to reach an economically and socially diverse population. Potential subjects who respond to the Rally posting will be informed of the purpose and procedures involved in the study, the location of the study and the remuneration involved for completing study visits by study staff as a part of the telephone prescreening. They will then be asked several screening questions regarding whether they are in the age range, sex, skin color, and lip assessment required for the study. If the potential subject is not within one of the screening criteria described, then study staff will explain that they are not qualified for the study and thank them for their time. If the screening criteria are met and the subject is interested in the study, study staff will take contact name, email and phone number and schedule the screening visit (phone/video call) with one of the licensed physician investigators listed on the protocol to go over the consent process.

V. SUBJECT ENROLLMENT

a. Method of Enrollment

All subjects will be subject to a telephone prescreening by study staff before scheduling the initial screening visit. All subjects who electronically sign an informed consent form (ICF) and are screened will be documented on a screening log. All subjects who qualify at the screening visit and who are enrolled in the study will be documented on the enrollment log. A note will be made in the source documentation verifying that the subject has willingly signed the ICF prior to participation in any study procedures.

b. Informed Consent Form (ICF)

One of the licensed physician investigators listed on the protocol will consent and inform the potential study subject of all aspects of the study and answer their questions. Sub-investigators may assist in the consent process. If the subject agrees to be a study subject, they will document consent in electronic form by signing the online informed consent form via Adobe eSign. Subjects who need more time to decide whether they would like to participate will have access to the electronic consent form and reschedule their informed consent call with the licensed physician investigator if they are interested in participating in the study.

The investigator is responsible for using a consent form that has been approved by the IRB/Mass General Brigham's HRC and is the most current version. If a new version of the consent form is approved by the IRB/MGB's HRC while a subject is still participating in the study and there are major changes to any component of the consent form, then the subject will be informed of the changes and, if the subject agrees to continue study participation, they should sign the updated form.

Electronic informed consent will be obtained by trained study staff prior to performance of any protocol-specific procedures.

VI. STUDY PROCEDURES

a. Study visits and procedures

Screening visit *(phone/video call)*

During the screening visit, the investigator will discuss with each subject the nature of the study, its requirements and its restrictions.
The following will be performed to determine eligibility:

- Review of inclusion/exclusion criteria
- Review of medical history, medications and demographics
- Lip examination

Subjects will be consented remotely by a licensed physician investigator, either over video conference or telephone, depending on subject's preference. Adobe eSign will be used to perform the consent signing, and a copy of the e-signed consent form will be kept in an encrypted file within the lab documents for this study. Subjects who qualify for the study will be scheduled for visit 1. Subjects who fulfill all inclusion and exclusion criteria may enroll and begin the Visit 1 procedures that same day.

Visit 1 *(1.5 hours)*

The following assessments will be performed during Visit 1:

- Review of inclusion/exclusion criteria
- Review of medical history, medications and demographics

The following procedures and evaluations will be performed:

- OCT imaging for microvasculature
- Three-dimensional imaging using the Cherry Imaging system (before and after the procedure)
- Digital photography of the lips and perioral area

Filler treatment of the lips with Restylane Kysse (if the subject has completely inverted lips the lip filler procedure will be optional and only imaging will be collected in visit 1)

- Lip volume assessment with Lip Fullness Grading Scale
- Subjects will be instructed how to report a pain score for first needle insertion and overall procedural pain. A visual 10 point pain scale will be used.

- Intravenous access will be placed to facilitate four blood draws to analyze serum oxytocin levels, all of which will occur on Visit 1 as follow: after photography and OCT, and before the filler procedure starts, immediately after the filler procedure, after the "mirror reveal" (when we show the lips to the subject using a mirror") minutes and 30- 35 minutes after the filler procedure has been concluded.
- The questionnaires: "FACE-Q^{TM} – satisfaction with outcome" and other questions will be applied right after the procedure to evaluate subject's satisfaction and about lip color and shape changes.
- Documentation of Adverse Events
- Video recording (15-30 seconds-video) from subjects' face and lips after the lip filler procedure. The video recording is optional.
- Upon completion of all treatments, the subject will be provided with aftercare instructions, remuneration and parking voucher if needed

Follow-up Call (*3-7 days after Visit 1, 30 minutes****)***
Study staff will call the subject 3-7 days after Visit 1 to check-in and inquire about any post-procedure pain or any side effects. If there are any negative side effects from treatment, they will be evaluated and treated in accordance with standard medical procedures.

Visit 2 *(2 weeks after Visit 1, 1 hour)*

Subjects will be asked to return 2 weeks after Visit 1. Visit 2 is expected to last up to 1 hour. The following procedures and evaluations will be performed:

- Three-dimensional imaging using the Cherry Imaging system
- OCT imaging for microvasculature
- Digital photography of the lips and perioral area
- Lip volume assessment with Lip Fullness Grading Scale (LFGS)
- Documentation of adverse events

- The questionnaires: "FACE-Q^{TM} – satisfaction with outcome" and other questions will be applied right after the procedure to evaluate subject's satisfaction and about lip color and shape changes.

b. Drugs to be used

Restylane® Kysse (Galderma Laboratories, L.P, Fort Worth, TX)

Restylane® Kysse injectable gel is a clear, colorless gel. It is a hyaluronic acid based dermal filler with local anesthetic lidocaine, which is FDA approved for lip augmentation and wrinkles around lips. The content of the syringe is sterile and each syringe comes with 1mL of injectable gel. In the event that a syringe contains material that is not clear or is cloudy, that syringe will not be used. Refrigeration of the product is not required, store at 25ºC/77ºF [9].

c. Devices to be used

Optical coherence tomography (OCT) Device (Thorlabs, Inc., Newton, NJ)

OCT imaging is performed using the commercially available, non-modified spectral-domain OCT scanner Telesto II (Thorlabs Inc.). It operates with a similar light exposure as FDA-approved systems such as the Vivosight Scanner (Michelson Diagnostics, Kent, UK) and Skintell (Afga HealthCare, Greenville, SC). The Telesto II consists of a broadband SLD light-source Class 1M (Class I FDA) at a center wavelength of 1300 nm and a maximum power of <50 mW. While the system achieves A-Scan rates of up to 76 kHz, note that the irradiation of the SLD is not pulsed.

Using a lens with an optical lateral resolution of 13 μm (LSM03, Thorlabs Inc. – spot size > 20 μm), we acquire images with an oversampling factor of 2× leading to voxel sizes of 6.5 × 6.5 × 3.5 μm3 and a field-of-view (FOV) of 6 × 6 × 3.5 mm3 (length × width × depth).

Although the system has not yet been approved by the FDA and is available in the United States for investigational use, its classification aligns with ANSI 136.1 class 1M and is thought to be incapable of causing harm.

This system was preferred over other commercial devices because of its detailed control of imaging parameters and the accessibility of the raw spectral data. This enables reproducible imaging as well as to obtain the imaging data needed to evaluate skin vascular characteristics.

Cherry Imaging Photography (Cherry Imaging Ltd, Yokneam, Israel)
The Cherry Imaging system is a 3-dimensional photographic imaging system designed to accurately measure aesthetic treatments to deliver objective patient data before and after aesthetic treatments. The handheld camera captures thousands of three-dimensional images of the face and/or body from multiple field views and angles that

are analyzed to provide 100-micron accuracy level data of the body and/or face for real-time evaluation of treatment results and traceability over time. The Cherry Imaging device will be used to take images of the face before the filler treatment takes place and during the last visit. No special lighting is needed for the imaging device, so the images will be taken and processed directly in the room where the filler procedure will take place. The Cherry imaging software has multiple capabilities, including the ability to calculate topographic changes, volume changes, and color changes in the skin.

d. Procedures/Interventions

Optical coherence tomography (OCT) imaging

The OCT imaging system will be cleansed with 70% isopropyl alcohol wipe in patient's presence prior to perform any imaging procedure. The subject's lips and surrounding skin at the intended OCT imaging sites will be cleansed with 70% isopropyl alcohol prep and allowed air dry thoroughly. First, OCT imaging will be performed in non-contact setting focused on the skin surface to image its profile. It takes about 1-2 minutes to properly position the patient and align OCT device per site and about 30 seconds per scan.
Secondly, a z-spacer (OCT-lmm03, ThorLabs, Inc., Newton, NJ) will be attached to the OCT probe and glycerol will be utilized for optical coupling (immersion) between the OCT spacer and the skin. In this setting, a sequence of three OCT angiographic images will be acquired. For imaging of the lips, a waterproof film polymer (i.e., Tegaderm) dressing will be used to minimize direct contact of the OCT spacer with the skin. Glycerol will be applied to the lips with a Q-tip. The middle portion of the bottom part of the lip will be imaged on each patient. One image will be taken. Then the OCT imaging device will be pressed onto the skin until the skin is blanched. When the skin is blanched, a second OCT image will be acquired. After the second image is acquired, the pressure will be held with the skin blanched for a further 60 seconds and then released. A third image will be taken about 10 seconds after pressure is released. The pressure required for blanching the skin will be measured and recorded. A brief test may be done near the imaging site prior to imaging in order to determine the blanching threshold pressure. The acquisition and saving time per image will be less than 1 minute. Taking these three images will allow assessment of the vascular density and vessel thickness as well as the vessel refilling characteristics after occlusion. If study staff operating the OCT imaging device decide the images are of too low quality or contain artifacts that would affect analysis, this procedure may be repeated on an area of the skin within close proximity to the original imaging location but at least 2-3 cm away from the original location. After finishing the OCT imaging procedures, the z-spacer will be detached and cleansed with 70% isopropyl alcohol wipe then allowed to air dry thoroughly prior to next use.

Cherry Imaging Photography
The Cherry Imaging system will be used to take images of the lips. The Cherry Imaging photography can take place at the MGH's Clinical Unit for Research Trials & Outcomes in Skin (CURTIS) or Translational Clinical Research Center (TCRC) since there is no special background or lighting condition needed to use the Cherry Imaging system.

Digital Photography

A DSLR camera will be used to take images of the lips and perioral area before the dermal filler procedure takes place during visit 1 and then at the second study visit. Subjects will be asked to stand with limited facial expression so that consistent, photographic images can be obtained.

Video Recording

Study's encrypted Iphone 12 (no chip inside, used only for the study) will be used by study staff (investigator or research coordinators) to take a video recording from the subjects' face/ reaction during the "mirror reveal". From 15 to 30 seconds of video recording will be taken from subject's face after a mirror is used to show them their "new lips". This will happen after the dermal filler procedure takes place during visit 1. This video will be optional and subjects will express if they allow this video recording or not in the consent form. Subject's reaction after the " mirror reveal" will be correlated to Oxytocin levels.

Restylane Kysse Lip Filler Treatment

After the OCT imaging, Cherry Imaging and digital photography, Restylane Kysse lip filler will be injected into the submucosal layer of the lips and subcutaneous layer of the perioral area. This hyaluronic acid filler formulation contains local anesthetic lidocaine so no topical anesthetic will be applied. The subject will be positioned in a seated position and the treatment area will be cleaned prior to injection. One syringe with 1mL will be injected to lips and perioral area in accordance with standard aesthetic lip filler practices. We aim to inject the full amount of one syringe, but upon the investigators discretion we may inject +/- 0.25mL as needed. The area will then be gently massaged with Vaseline to ensure the dermal filler gel is distributed evenly [9]. The subject will experience sharp pain and mild pressure from initial injection, which will subside with subsequent insertions as the lidocaine numbing takes effect. Standard of care for aesthetic lip filler treatment will be followed.

Blood Draw

Four blood draws to analyze serum oxytocin levels, all of which will occur on Visit 1 as follow:

- After photography and OCT, and before the filler procedure starts, an intravenous access will be placed and approximately 3 cc of blood will be drawn into EDTA tubes containing Aprotinin supplied by the Brigham Research Core lab.
- Immediately after the filler procedure, approximately 3 cc of blood will be drawn into EDTA tubes containing Aprotinin supplied by the Brigham Research Core lab.
- after the after the "mirror reveal" (when we show the lips to the subject using a mirror approximately 3 cc of blood will be drawn into EDTA tubes containing Aprotinin supplied by the Brigham Research Core lab.

- 30-35 minutes after the filler procedure has been concluded, a final approximate of 3 cc of blood will be drawn into EDTA tubes containing Aprotinin supplied by the Brigham Research Core lab.

Each sample will be allowed to clot for 30-60 minutes at room temperature to prepare serum, then centrifuged at 1600 x g for 15 minutes at 4°C by study staff, and the serum will be transferred to plastic tubes.
The samples will be placed on dry ice and hand-delivered by the research staff to the Brigham Research Core lab or stored at -80 degrees freezer located at the MGH Navy Yard in the Manstein lab when immediate delivery is not possible.
The Brigham Research Core lab will process the ELISA assay to evaluate oxytocin levels.

e. Data to be collected and when data is collected

Study data will be collected during each study visit. Study data to be collected includes OCT imaging (various images as described above, visit 1 and 2), digital photography and Cherry Imaging of the lips and perioral area (visit 1 and 2), visual assessment of treated area (visit 2), video recording (optional) from subject's reaction after the mirror reveal, Lip volume assessment with Lip Fullness Grading Scale (screening visit, visit 1 and 2), pain assessment of initial injection and overall procedure (visit 1), blood samples (visit 1) and satisfaction questionnaire answers (visit 1 and 2). Medical history and medications will be documented at visit 1. There are no other biological data or health information to be collected in this study.

Information about side effects in the treatment area or any adverse events will be collected during the follow-up call and visits.

f. Remuneration

Subjects will be paid $50 for completing all imaging activities and filler treatment during visit 1, and $100 for completing visit 2. The total remuneration amount is $150 upon completing the study. We will provide a parking voucher at MGH main campus for each visit upon request.

We will be using an approved, outside vendor (Forte Research) to make these payments via a reloadable credit card-based system, called Forte Payments. This secure system is similar to a gift card or credit card.

If subjects are paid by this system, they will be given a Forte Payments Via card (which is just like a debit card) when subjects enroll in the study. Once the card is activated, the study team will add a payment after each paid visit completed by the subjects. The payment should be available within one (1) business day. Research staff will not know where subjects spend the money. You may use the card anywhere Visa cards are accepted, such as at a grocery store.

We will need to collects subjects' Social Security number in order to make these payments, and it will be shared securely with the company that runs the card-based system. Payments like this are considered taxable income. If subjects receive more than $600, the payment will be reported to the IRS as income by the hospital.

VII. BIOSTATISTICAL ANALYSIS

a. Study Variables

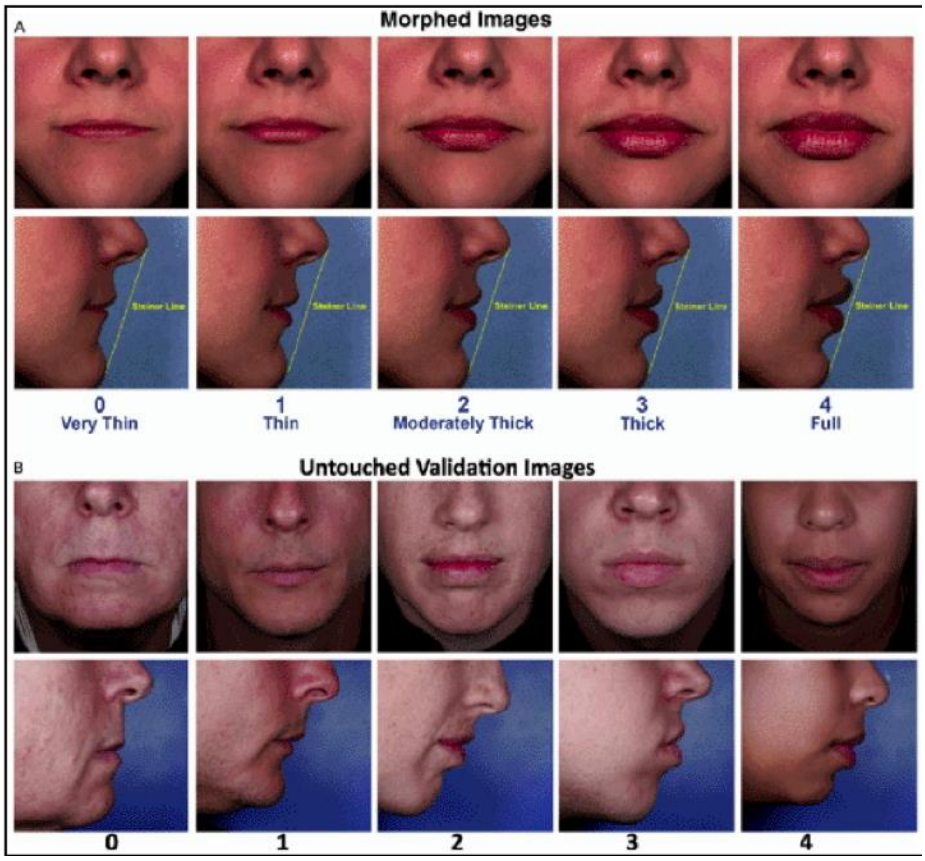
Aesthetic Procedure Improvement Analysis

The Global Aesthetic Improvement Scale (GAIS) is a five-point scale used to assess effectiveness of aesthetic procedures comparing pre-treatment and post treatment. This scale ranges from 1= exceptional improvement, 2 = very improved patient, 3 = improved patient, 4 = unaltered patient, 5 = worsened patient [11, 13].

	Degree	Description
1	Exceptional improvement	Excellent corrective result
2	Very improved patient	Marked improvement of the appearance, but not completely optimal
3	Improved patient	Improvement of the appearance, better compared with the initial condition, but a touch-up is advised
4	Unaltered patient	The appearance substantially remains the same compared with the original condition
5	Worsened patient	The appearance has worsened compared with the original condition

Lip Volume Analysis

Lip Fullness Grading Scale (LFGS) will be used to assess the lip volume change from pretreatment compared to post treatment. This photonumeric rating scale ranges from 0 = very thin; 1 = thin; 2 = moderately thick; 3 = thick; and 4 = full. Improvement of lip volume is defined as ≥1 increase at post-treatment compared to pretreatment [11, 14].



Pain Scoring Analysis

The Wong-Baker FACES Pain Rating Scale will be used to score subject pain from the first insertion of dermal filler and the overall pain from the procedure.

Wong-Baker FACES® Pain Rating Scale

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b. Study Analyses

OCT analysis

OCT imaging is performed using the commercially available spectral-domain OCT scanner TELESTO II (Thorlabs Inc., Newton, New Jersey). It operates at a central

wavelength of 1,300 nm with a maximum output power of less than 10 mW. Using a lens with an optical lateral resolution of 13 µm (LSM03, Thorlabs Inc.), we acquire images with an oversampling factor of 2× leading to voxel sizes of 6.5 × 6.5 × 3.5 µm3 and a field-of-view (FOV) of 6 × 6 × 3.5 mm3 (length × width × depth), acquired at an A-Scan rate of 76 kHz.

The imaging data is further processed and analyzed by custom made software tools implemented in MATLAB 2016b (MathWorks Inc). All OCT image data files will be labeled with the study subject number assigned to each subject upon consent. No identifying information will be used in labeling the image data files and the files will be stored on Partners OneDrive.

With angiographic image post processing, the capillary layer, typically in between 70 − 400 µm depth in skin, is displayed and converted to 2-dimensional images as maximum intensity projections. These 2-dimensional images are further segmented by an automatic, customized software and quantitative metrics such as vessel area, vessel length, vascular network complexity (fractal dimension) and vessel curvature are assessed. These characteristics of the capillary network are further used for inter- and intra-cohort investigations.

Cherry Imaging Photography Analysis

The Cherry Imaging analysis software contains distinct algorithms that process the generated three-dimensional images to calculate volume in cubic centimeters, precise color using saturation and hue, roughness detecting local variations in height/depth, and a ruler function that measures the distance between two distinct points. These will all be employed to analyze photos of lips, lip redness, roughness and volume.

DSLR Camera Photography Analysis

Standard photographs will be blindly reviewed by a physician and assessed using the Lip Fullness Grading Scale and Global Aesthetic Improvement Scale.

Video Recording

Videos will be blindly reviewed by a physician and assessed using the following grading scale (this grading scale is based on the model of answers that are given to subjects in the questionnaire):

Very satisfied	Somewhat satisfied	Somewhat dissatisfied	Very dissatisfied

All video recording data files will be labeled with the study subject number assigned to each subject upon consent. No identifying information will be used in labeling the video data files and the files will be stored on Partners Dropbox. The videos will be stored at Partners Dropbox until 7 years after the end of this study when all the copies will be permanently deleted from this drive.

Only study staff will have access to the videos (for storage purposes- like research coordinators or for assessment - investigators) unless the subject authorizes, by signalizing it in the consent form, the use of their video recordings for the following purposes:

- medical or pharmaceutical publications, or presentations in conferences
- educating health care professionals (such as physicians and nurses) through electronic materials (iPads), brochures and the healthcare professional website
- patient-focused websites and patient-handouts / industry ads

If the subject agrees (in the consent form) with the use of her video recording for the purposes cited above the video will be shared with pharmaceutical companies (ex. Galderma- Lip filler producer) or will be shared with other doctors during lectures in medical conferences.

Oxytoxin

The Brigham Research Core lab will process the ELISA assay to evaluate oxytocin levels per the Oxytocin EIA kit which is a colorimetric competitive enzyme immunoassay kit, with very low reactivity with vasopressin.

c. Statistical Methods

The study is intended to gather empirical data about the volume, color, texture and microcirculation change in the lips and perioral area; and pain assessment of treatment. Descriptive statistics will be collected to present quantitative descriptions of changes in lips and perioral area from pretreatment compared to post-treatment.

VIII. RISKS AND DISCOMFORTS

a. Complications of surgical and non-surgical procedures

Subjects might encounter an experience of discomfort during OCT imaging procedure due to the application of pressure to occlude blood vessels in the skin. There will be no harm caused by vessel occlusion and study staff will explain the procedure to subjects prior to taking the OCT images. There are no known adverse effects of the three-dimensional imaging system (Cherry) other than psychological distress from seeing a high-resolution photo that may highlight imperfections. This is no different than routine professional photography.

The IV access can cause pain during its placement and any infection, thrombophlebitis or other adverse outcome will be treated according to standard medical care and recorded in the Adverse Event Log.

b. Drug side effects and toxicities

Possible side effects of Restylane Kysse dermal filler include redness (very common)*, pain/tenderness (very common), swelling (very common), lumps/bumps (very common), bruising (very common), itching (very common), or discoloration (very common). Potential risks included allergic reaction (rare but serious), infection (rare but serious), scarring (rare but serious), skin ulceration (rare but serious), ischemia (rare but serious), infarction (rare but serious) or change in skin tone (very common) [9].

Dermal fillers in the lips and perioral area are considered to be low risk but rare instances of localized skin necrosis are possible. In this case, if there are symptoms of occlusions, intense pain, or purple patches upon injection, we will have hyaluronidase (FDA approved for vascular occlusion necrosis) available in clinic.

A subject could have an allergic reaction to glycerol, Tegaderm or dermal filler (rare but serious). In this case, the allergic reaction would be recorded in the Adverse Events Log and would be monitored and treated by study staff in accordance with standard medical procedures.

*Please refer to the National Cancer Institute frequency categories listed below for reporting of possible side effects:

a. Very Common (more than 1 out of 10 people)
b. Common (between 1 and 10 out of 100 people)
c. Uncommon (between 1 and 10 out of 1,000 people)
d. Rare but Serious (less than 1 out of 1,000 people)

c. Device complications/malfunctions

Telesto II OCT imaging device (Thorlabs, Newton, NJ)

The Telesto II system is controlled and triggered by an external PC workstation. In case the OCT acquisition software (ThorImage, Thorlabs Inc.) or the operating system malfunctions, the operator will reboot the system.

Cherry Imaging, Version 19.08.3322 (Yokneam, Israel)

The Cherry Imaging system is a photographic system that captures patient images using standard lighting conditions with no special positioning required. The images are analyzed using Cherry Imaging algorithms to create volumetric images of the photographed area. We expect no device complications or malfunctions due to the Cherry Imaging system.

d. Psychosocial (non-medical) risks

There is a potential risk of loss of privacy. We will protect privacy by labeling samples, information, and data files only with a study subject number code, and keeping the key to the code in a password protected database.

IX. POTENTIAL BENEFITS

a. Potential Benefits to Subjects

Subjects who participate in this study will receive Restylane Kysse dermal filler treatment of the lips, which may improve the appearance of their lips.

b. Potential Benefits to Society

Information gathered from this study may improve the understanding of the pathological alteration of intrinsic aging to lip microvasculature and changes in color, roughness and/or appearance of lips before and after a lip filler procedure. This study may also aid in understanding pain tolerance with the lip filler procedure to improve pain management techniques in the future as needed.

X. MONITORING AND QUALITY ASSURANCE

a. Independent monitoring of source data

Experienced study personnel (study monitor) who are not assigned to complete procedures of this study will conduct monitoring after the first subject is enrolled and periodically thereafter. The monitor will be responsible for confirming the completion and correctness of the study procedures as well as record collection and keeping.

b. Safety monitoring

Prior to enrollment, subjects will be screened for eligibility; at which time a complete medical history, including a baseline assessment of the subject's lips will be done. Evaluations will be ongoing throughout the study to detect adverse events and changes in existing medical conditions.

At any time after enrollment, a subject may be discontinued. Reasons for discontinuation of a subject from the study will include, but may not be limited to, the following:

1. Subject is found to be intolerant to a required study procedure at any time point.
2. Subject is noncompliant with protocol restrictions and requirements.
3. Subject develops an intercurrent illness that would, in the judgment of the investigator, affect assessments of clinical status to a significant degree.
4. Subject becomes pregnant while participating in the study.
5. Subject enrolls in another investigational study.
6. Subject requests to withdraw from the study.
7. The study staff decides to suspend or terminate the study.

If possible, a final set of assessments will be performed on all subjects who end their participation prior to study completion.

c. Outcomes monitoring

The study will be conducted in accordance with applicable regulations and Good Clinical Practice Guidelines. Keeping files locked with access limited to study staff will ensure confidentiality and data integrity.

d. Adverse Event Reporting

Definition
Adverse Event (AE) is any untoward medical occurrence in a subject that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign, symptom, or disease temporally associated with the use of an investigational product, whether or not related to the investigational product.

Serious Adverse Event (SAE) is any untoward medical occurrence that:

- Results in death
- Is life-threatening
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability or incapacity
- Is a congenital anomaly/birth defect
- Is another medically important condition

Reporting and Documenting Adverse Events
All untoward medical occurrences that occur after the subject signs a consent form will be documented as an AE. The Investigator will ensure that all events that occur during the study period are recorded. All AEs will be followed until resolution or until, in the Investigator's judgment, they are chronic and stable. If an emergency situation should occur, appropriate medical measures should be taken to stabilize the subject.

Documentation of AEs includes: date and time of onset and resolution of AE, intensity, frequency, seriousness, related interventions and outcome. The Investigator will also evaluate the probability of a causal relationship of the AE to the study treatment as being: "definite, probable, possible, unlikely, or unrelated." Intensity of adverse events will be graded as mild, moderate, or severe according to the following criteria:

- Mild: symptoms that are easily tolerated and transient in nature with minimal or no impairment of normal activity
- Moderate: symptoms that are poorly tolerated, are sustained, and interfere with normal activity
- Severe: symptoms that are incapacitating and render the subject unable to work or participate in many or all usual activities

All SAEs will be reported to the IRB according to the IRB's requirements.

Adverse events will be reported to the PHRC as described in the PHRC policy on Unanticipated Problems Involving Risks to Subjects or Others Including Adverse Events, which can be found on the Partner's Research Navigator website.

XII. Data Management

a. Data collection

All study data will be collected during both study visits. Study data to be collected includes clinical photography, Cherry images, OCT images and qualitative patient responses to treatment and laboratory results. Pain scores will be recorded on the case report form for visit 1, LFGS for screening visit, visit 1 and 2. Subjects' answers for satisfaction evaluation questions will be also recorded. All physical documentation and IRB correspondence will be stored in study binders maintained in restricted lab space only accessible by study staff and members of the Manstein Lab. Digital data including photographs will be deidentified and stored on MGB computers. Any identifiable information in photographs such as eyes and tattoos will be blacked out and deidentified. All study documents containing PHI will be password encrypted, including the enrollment log and identification key. An encrypted external hard drive will be used to store the data as a backup.

b. Record retention

The Investigator or designees will retain all study records in accordance with the test facility's SOP's.

XIII. IRB Review and Approval

The study will not begin prior to the receipt of written confirmation of approval by the IRB and any relevant regulatory authority. It is the responsibility of the Investigator to obtain the IRB approval (per the U.S. Code of Federal Regulations, Title 21, Part 56 and applicable ICH guidelines) for the protocol, amendments, informed consent, subject information sheet, questionnaires, and advertising materials used to recruit study subjects, if appropriate.

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Appendix A: Study Schema

	Screening	Visit 1	Follow-up Call	Visit 2
Study Visit	<30 days	Day 0	3-7 days after Visit 1	2 weeks after Visit 1
Informed Consent	X			
Inclusion/Exclusion	X	X		
History/Demographics	X			
Review side effects/check-in			X	
Investigator Assessments		X	X	X
Digital Photography		X		X
3D Cherry Imaging		X		X
OCT Imaging		X		X
Filler Treatment		X		
Questionnaires		X		X
LFGS	X	X		X
Con Meds/Adverse Events		X	X	X
Blood Draw		X		