A single center, prospective, blinded study to evaluate the efficacy and safety of a tripeptide/hexapeptide topical when used with Er:YAG hybrid laser for the treatment of acne scars.

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1. PURPOSE OF STUDY

Purpose:

To evaluate the efficacy of a tripeptide/hexapeptide topical (Alastin Regenerating Skin Nectar with TriHex Technology®) in wound healing and scar reduction following Erbium:YAG hybrid resurfacing laser for acne scarring compared to the standard of post-procedure care consisting of a bland moisturizer.

Specific Aims:

1. Demonstrate the safety and tolerability of the tripeptide/hexapeptide topical used when added to Erbium:YAG laser.
   - Record adverse effects
2. Investigate the performance of a tripeptide/hexapeptide topical compared to the standard of care in patients undergoing Erbium:YAG hybrid laser.
   - Change in skin erythema (colorimeter)
   - Change in skin barrier function (transepidermal water loss [TEWL])
   - Improvement in scarring measured by clinical photography, Global Aesthetic Improvement Scale (GAIS)
3. Demonstrate histologic differences in patients treated with tripeptide/hexapeptide topical vs standard of care after undergoing Erbium:YAG hybrid laser.
   - Changes in collagen and elastin deposition (pre- and 6 months post-treatment)
4. Evaluate subject satisfaction with self-assessment surveys

Hypothesis:

We hypothesize that pre and post-procedural care with the tripeptide/hexapeptide topical will improve the cosmetic outcome (erythema, barrier function, scar improvement) and reduce recovery time when compared to current standard of care in patient's undergoing Erbium:YAG hybrid laser. The proposed mechanism of improvement is through increased stimulation of collagen and elastin synthesis (to be measured on histology and TEWL).

2. BACKGROUND AND RATIONALE

Acne and subsequent scarring has been linked to emotional debilitation, embarrassment and anxiety.[1] The prevalence of acne scarring is 1-11% of the general population and can affect up to 95% of patients with acne vulgaris.[2,3,4]

Three primary acne scars include icepick (<2 mm, deep with tracts to dermis), rolling (circular or linear, >4mm, gently sloped edges merging with normal skin) and boxcar (wide based 1.5-4mm diameter; shallow <0.5mm, deep >0.5mm).[5] Various medical and procedural modalities have been employed to reduce acne scarring. Medical management includes retinoids,

topical/injectable steroids, silicone dressing. Surgical management includes punch excision, elliptical excision, punch elevation, skin grafting, subcision and debulking. Procedural management includes lasers, cryosurgery, electrodessication, radiation, chemical peels, microneedling and dermabrasion.[6,7]

Amongst laser therapy, ablative modalities have been shown to reduce the appearance of shallow boxcar and ice-pick scars.[8] Side effects of ablative laser therapy include delayed healing, infection, scarring, erythema, acne, milia, edema and dyspigmentation. In order to improve side effect profile, fractional photothermolysis has become a reasonable option for resurfacing. It ablates tissue in a columnar fashion, creating microscopic thermal zones and leaving areas of native epidermis and dermis to provide for collagen remodeling and neogenesis. After fractional ablation, basal keratinocytes begin to re-establish a continuous basement membrane with 24 hours and complete epidermal regeneration by 7 days.[9] Graber et al noted a 7.6% complication rate (of 961 patients) after fractional ablative laser therapy for photodamage. The most frequent complications were acneiform eruptions and herpes simplex virus outbreaks (both approximately 1.7%).[10]

The standard of post-procedural laser care is varied but has included vitamin E, vitamin C, aloe vera, bovine collagen, petrolatum and bland creams. Data to support the superiority of one over another is limited.[11] More recently, newly formulated topical systems targeted at optimizing wound healing have been studied. Tripeptide and hexapeptide (TriHex Technology™, Alastin Skincare Inc) was designed to promote extracellular matrix remodeling for pre- and post-procedural care. It is thought to increase tropoelastin and procollagen production resulting in elastogenesis, collagenesis promoting overall wound repair.

Wilson et al showed tripeptide/hexapeptide post-procedural care was associated with a shortened downtime and decreased symptoms following laser resurfacing of the face.[12] Patients reported a significantly higher level of satisfaction compared to controls.[12] Robinson et al showed superior healing at post-procedure day 4 (blinded investigator and patient impression), significantly better skin tone, and higher levels of patient satisfaction compared to the control regimen.[13]

3. ADMINISTRATIVE ORGANIZATION

University Dermatology Associates within the University of Rochester Medical Center, located at Collegetown Dermatology.

4. STUDY DESIGN

This is an investigator initiated and patient-blinded, randomized clinical study comparing tripeptide/hexapeptide topical (Alastin Regenerating Skin Nectar with TriHex Technology®) to a bland moisturizer after laser resurfacing for acne scars.

Study outcomes:

1. Demonstrate the safety and tolerability of the tripeptide/ hexapeptide topical system when added to standard of care Erbium:YAG hybrid laser.
   - Record adverse effects
2. Investigate outcomes of tripeptide/hexapeptide topical compared to standard of care in patient undergoing Erbium:YAG hybrid laser.

- Change in erythema (colorimeter)
- Change in barrier function (Trans Epidermal Water Loss-TEWL)
- Improvement in scarring (clinical photography, Global Aesthetic Improvement Scale-GAIS)

3. Demonstrate histologic differences in patients treated with tripeptide/hexapeptide topical vs standard of care after undergoing Erbium:YAG hybrid laser.
   - Changes in collagen and elastin deposition (pre- and 3 months post-treatment)
4. Evaluate subject satisfaction with self-assessment surveys

Subjects will be scheduled for an initial visit at the dermatology clinic, consented and screened. It will be required that subjects be able and willing to comply with the treatment regimen and follow-up obligations. Subjects will be asked to complete an initial personal assessment rating the severity of their acne scarring (1 = very mild, 2 = mild, 3 = moderate, 4 = moderately severe, 5 = severe) and evaluation of the psychosocial impact of their condition. There will be a window of +/- 5 days for each visit to accommodate for subject scheduling.

Baseline photographs with a digital camera will be taken. Patients will be randomized into either treatment or control groups. The treatment group applies tripeptide/hexapeptide topical (Alastin Regenerating Skin Nectar with TriHex Technology®) and the control group applies a bland moisturizer (Cetaphil moisturizer, standard of care). Both groups will use the same gentle cleanser and SPF-30+ sunscreen. Subjects will be assigned a de-identified number and then randomized (random number generator) to control or treatment groups. The topicals will be in unlabeled bottles so individuals will be blinded to what group they are in.

A blinded investigator or research coordinator will measure TEWL, Colorimetry, Biopsy, Global Aesthetic Improvement Scale (GAIS) and the Goodman and Baron qualitative scale (attached document). Adverse events will be assessed from the time the subject signs consent (day -14) through the completion of the follow up period (day 90). Adverse events specifically screened for will include pain, edema, erythema, petechiae, or dyspigmentation. The Erbium:YAG hybrid laser procedure will be performed by Dr. Mara Weinstein Velez and a sub-investigator(s). Laser settings will be adjusted according to Skin Type to reduce risk of dyspigmentation. The investigators will use the laser according to standard of care practice and settings.

Two weeks prior to the laser procedure, all subjects will wash their face twice daily with gentle cleanser followed by application of either the Alastin Regenerating Skin Nectar with TriHex Technology® or Cetaphil moisturizer. This will be followed by SPF 30+ sunscreen. Two subjects from control and experimental groups will be selected for a biopsy of representative areas on the face at baseline (day -14) and at study conclusion (day 90). All subjects will be asked if they consent to the biopsy procedure until their cohort has two participants willing to participate in the biopsy portion of the study. Once each cohort has two participants in the biopsy portion, no further subjects will be asked to participate in the biopsy portion.

Sixty minutes prior to the laser treatment (day 0), topical anesthesia (tetracaine 7% with lidocaine 23% ointment) will be applied to subjects. Subjects will then undergo Er:YAG hybrid laser resurfacing to the face in a standard of care fashion. Following the procedure, either the

Alastin Regenerating Skin Nectar with TriHex Technology® or Cetaphil moisturizer will be applied to the face by the subject.

Prior to the second laser treatment (day 30), the same topical anesthesia will be applied. 60 minutes later, the subjects will undergo a second session of Er:YAG hybrid laser resurfacing to the face. Following the procedure, the randomized topical product will be applied to the face by the subject.

On post-procedure day 4 and day 34, patient photographs will be taken, surveys completed, and investigator assessments taken. On all other remaining post-procedure days (ie. Days 1-29, 31-90) subjects will be asked to wash their face twice daily with gentle cleanser followed by application of the randomized topical product and SPF 30+ sunscreen.

At each evaluation, subjects will be asked to complete a patient self-satisfaction scale (0-10) regarding acne scars, erythema, and general skin condition (see attached). Any adverse event will be recorded. A blinded investigator will then evaluate the skin surface for erythema (colorimeter), Trans-Epidermal Water Loss (TWEL) and overall appearance (Global Aesthetic Improvement Scale-GAIS). At the final visit, subjects will be asked to complete a supplemental questionnaire that allows them to share personal perceptions of scar improvement. Photo representation will be provided to investigators to provide a baseline training for using the Global Aesthetic Improvement Scale.

At the end of the study, a blinded investigator will select the post-treatment photograph and quantify the degree of improvement seen in the image as poor (0-24%), good (25-49%), very good (50-75%), or excellent (76-100%).

Histologic analysis will be performed by a dermatopathologist blinded to treatment group. Specimens will be examined and compared with their baseline levels to assess for differences in levels of collagen and elastin.

4.1. SUBJECT POPULATION

We plan to enroll a total of 10 subjects (male or female), age >18, with evidence of grade II-III acne scars on the face (mild to moderate) as determined by the Goodman & Baron qualitative global acne scar grading system. This system was chosen given its widespread acceptance as a simple and effective means of normalizing objective appearances among patients. Individuals must have decision making capacity, and be able to read, write and understand English. Subjects will be identified from routine College Town Dermatology clinic visits (those that are being seen for acne or non-acne related visits). Subjects who are interested in the trial will be offered a screening visit.

Exclusion criteria: treatment to face with any energy device within 6 months, tanning within 7 days, dermabrasion or chemical peel within 3 months, use of systemic retinoids within 6 months, history of hypertrophic scarring or keloids, use of systemic steroids within 6 months, or use of topical products with retinoid, alpha-hydroxyacid, salicyclic acid, vitamin C or E within 14 days.

We will not enroll patients of the "vulnerable population" group (ie. Children, pregnant women,

prisoners, or decisionally impaired adults).

This study will adhere to the guidelines from the University of Rochester in performing human research subject research in the COVID-19 Era. The guidelines can be found at: https://www.urmc.rochester.edu/coronavirus/coronavirus-research/guidance-for-researchers/human-subjects-research.aspx

4.2. STUDY INTERVENTIONS

-Subject will apply Alastin Regenerating Skin Nectar with TriHex Technology® or Cetaphil followed by broad-spectrum sunscreen with SPF 30. These are over counter creams. They do not undergo FDA testing, they are not prescription medications.

-Accountability, storage, access, control of drug(s): the creams will be given to the subjects to take home. These over the counter topical products do not need to be refridgerated or stored in a special manner.

-Blinding/labeling/preparation of agents: patients will be provided a non-labeled container so as not to know which cream they are applying (Alastin vs. Cetaphil). The sunscreen will be universally applied by all patients.

-Device(s) being used: Halo™ Hybrid Fractional Laser (Sciton, Inc. Palo Alto, CA) delivering non-ablative (1470nm) wavelengths and ablative (2940nm) wavelengths. This will be performed in a standard of care manner.

-Status of device: Is a class II device that is FDA approved for treatment of wrinkles and scar reduction (acne scars).

-description of how device(s) will be used: Halo™ Hybrid Fractional Laser will be programmed according to skin type. Laser settings will be chosen by the PI, in a standard of care manner, according to the manufacturers recommended settings.

5. INCLUSION AND EXCLUSION CRITERIA

Inclusion criteria: male of females, age 18 and older, with evidence of grade II-III acne scars on the face (mild to moderate) as determined by the Goodman & Baron qualitative global acne scar grading system.

Exclusion criteria: treatment to face with any energy device within 6 months, tanning within 7 days, dermabrasion or chemical peel within 3 months, current use of systemic retinoids, keloidal scaring in the treatment area, use of systemic steroids within 6 months, or use of topical products with retinoid, alpha-hydroxyacid, salicylic acid, vitamin C or E within 14 days.

We will not enroll patients of the "vulnerable population" group (ie. Children, pregnant women, prisoners, or decisionally impaired adults).

6. RECRUITMENT METHODS

Subjects will be identified during regular clinic visits at College town Dermatology (acne related or non-acne related). Patient identified by the primary investigator or sub investigators will be offered a screening visit.

7. CONSENT PROCESS

Informed consent will be obtained by the principal or sub investigators; before any study enrollment or implementation is completed. Informed consent will be obtained by a sub-investigator or research coordinator (not directly involved in the patient's routine care). This will minimize the potential for coercion or undue influence. Potential subjects will be given the opportunity to take the consent form home. They may call our office to enroll or with questions anytime. All subjects will be provided with a signed copy of the consent form. At any time the subject may withdraw his or her consent.

8. STUDY PROCEDURES

An investigator will review inclusion and exclusion criteria for each subject. If subjects do not meet the criteria, they will be informed and released from the study. Patient demographics and patient satisfaction measures will be provided directly by patient.
Patients will be assigned a number and a random number generator will distribute patients to treatment or control groups.
Duration of individual's participation in the study will be from the initial screening visit to 3 months after initial treatment.
Please see the tabular representation of the study assessments and activities attached.

Once the study is complete, patients will be given access to their Global aesthetic improvement scale.
Any incidental findings that might have health consequences for the individual subject will be immediately and openly disclosed to the subject.

9. RISKS TO SUBJECTS

Risks from using the topical and laser treatment include but may not be limited to: skin redness, acne, dryness, roughness, bronzing, swelling, flaking, itching, scabbing, pinpoint bleeding, bruising, scarring, blistering, infection, rash, peeling, skin darkening or lightening, and localized pain.

Risk from the biopsy include: scarring, bleeding, infection, or loss of nerve function.

10. POTENTIAL BENEFITS TO SUBJECTS

There may be no benefit from being in this study. Possible benefits from being in the study may include: improved appearance and decrease in the size of acne scars.

11. COSTS FOR PARTICIPATION

There are no direct costs associated with participation in this study. The insurance carrier and/or subject will not be billed for study visits or tests that are part of this research. The topical skin care will be provided by Alastin Skincare Inc. free of charge to the subject. The laser treatment will also be free of charge to the subject. The department of dermatology will cover the cost of the laser treatment.

All study visits (including pre-treatment and follow-up visits), laser therapy and procedures

(biopsies, slides and studies of collagen and elastin expression) will be covered by the study team and department of dermatology. If there were to be adverse events or complications as a result of this study the care rendered would be considered non-study healthcare as a (non-study) patient in standard fashion. These visits, and any further workup or testing, would be billed to commercial insurance carrier in a standard fashion. The coverage of these visits cannot be pre-determined by the study team and may result in certain out of pocket costs at the discretion of subject's insurance.

12. PAYMENT FOR PARTICIPATION

There is no payment for participating in this study.

13. SUBJECT WITHDRAWALS

The decision to participate in this research study is entirely voluntary. Participation may be terminated by the study doctor or study sponsor at the discretion of the primary investigator, for example, if a participant experiences a serious adverse event.

Subject may refuse to participate in this investigation or withdraw consent and quit this study without penalty and without affecting the ability to receive medical care at the University of Rochester Medical center.

In order to be included in the study data analysis, subjects must complete the laser treatments and pre-, post- care. If a subject withdraws or is withdrawn from the study, incomplete data will not be used for analysis. If a subject withdraws from the study, then a new subject may be recruited.

14. PRIVACY AND CONFIDENTIALITY OF SUBJECTS AND RESEARCH DATA

Subjects will be assigned a depersonalized number and referred to that number for the remainder of the study. All data will be stored on a password encrypted shared drive on the University computer network. Pathology samples (4 subjects) will be stored and processed in the usual fashion. Labeled and stored in formalin for processing. It will not leave the university.

Data will be stored for 3 years after completion of the study, then discarded confidentially. The principal and subinvestigators will have access to the data and samples. Data will be shared with the following entities in a de-identified manner:

-Alastin Skincare Inc:

- Pathology
- photographs of acne scars prior and following treatment
- measurements of scars prior and following treatment
- side effects secondary to treatments.
- Photographs will be de-identified

15. DATA / SAMPLE STORAGE FOR FUTURE USE

Data will only be stored for up to 3 years after study completion. It will be stored in a de-identified manner and in a password encrypted, shared, university network server. Data will only be accessible by the principal and sub investigators.

16. DATA AND SAFETY MONITORING PLAN

Both the principal investigator and sub investigators will monitor, identify, report and manage any adverse events for the study. All adverse events will be assessed by the PI and recorded in the subject's study chart. The PI will report these adverse events according to the requirements of the University of Rochester's Research Subjects Review Board. The PI will report suspected serious adverse events (SAEs) to an independent, objective physician within 24 hours. He/she will independently determine whether the adverse event is related to the study treatment. The PI will report these SAEs, whether study treatment related or not, to the RSRB (IRB) through their electronic system, CLICK, within 24 hours of learning of the event. This will allow us to ensure the safety of our patients during the study period.

17. DATA ANALYSIS PLAN

A sample size of 10 will allow the investigators to adequately treat and compare placebo to treatment groups 5 vs. 5 assuming everyone stays enrolled in the study. This is a pilot study and therefore our goals are to obtain preliminary data with which we can then work to design a larger study.

We will work with a statistician in order to accurately and efficiently put together data that is collected based on our endpoints described above.

18. REFERENCES

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Appendix 1. Schedule of events

Visit Day (across	Screening day −(14) (+/-5 days)	Txmt #1 Day 0 (+/-5 days)	Day 4 (+/-5 days)	Txmt #2 Day 30 (+/-5 days)	Day 34 (+/-5 days)	Day 90 (+/-5 days)
Obtain Informed consent	X					
Confirm Eligibility	X					
Demographics	X					
Medical History	X					
Concomitant Medications	X					
Urine pregnancy	X	X		X		
Enroll/Randomize	X					
Vitals	X	X	X	X	X	X
Photos	X	X	X	X	X	X
TEWL	X	X	X	X	X	X
Colorimetry	X	X	X	X	X	X
Biopsy (4 subjects)	X					X
Patient Subjective Satisfaction	X	X	X	X	X	X
Global Aesthetic Improvement Scale (GAIS)		X		X		X
Goodman and Baron Qualitative Scale	X	X		X		X
Adverse Events Evaluation	X	X	X	X	X	X
Er-YAG Hybrid laser treatment		X		X		
Apply randomized topical cream	X	X	X	X	X	X