Human Platelet Extract (HPETM) is an effective topical cosmetic product for anti-aging and facial rejuvenation.

NCT05457491

4/19/2022

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General Study Information

Principal Investigator: Saranya P. Wyles, M.D., Ph.D.

Study Title: Novel Antiaging Regenerative Skin Care Regimen Containing Human Platelet Extract (HPE)

Protocol version number and date: Version 06 – April 19, 2022

Research Question and Aims

Hypothesis: Human Platelet Extract (HPE™) is an effective topical cosmetic product for anti-aging and facial rejuvenation.

Aims, purpose, or objectives:

The objective of this cosmetic study is to evaluate the role of HPE on aging skin appearance.

To assess the effect of HPE on fine lines/wrinkles, skin tone/texture, erythema, and pigmentation.

To compare the effect of HPE to market-competitor topical antioxidant (Skinceuticals™ CE Ferulic).

Background (*Include relevant experience, gaps in current knowledge, preliminary data, etc.*):

Skin aging is caused by free radical destruction of proteins, lipids, deoxyribonucleic acid (DNA), ribonucleic acid (RNA), organelles, and other vital structures. Under normal conditions, free radical damage is repaired by antioxidants, but these unfortunately decline with age, increasing the likelihood of continuous damage. Oxidative stress occurs when there is a loss of balance between free radical damage and cellular antioxidant self-repair [1]. This balance favors oxidation as small, nontoxic amounts of reactive oxidative species are required for cellular communication, immune system function, and control of the cell life cycle, which leads to beneficial cellular adaptation and balance. The skin also receives damage from free radicals generated by external factors, such as air pollutants, smoking, and changes in humidity and temperature [2]. These extrinsic causes of skin aging become more pronounced on sun-exposed areas such as the face and dorsal hands.

The visibility of the skin represents a unique organ system that facilitates inspection following application of topical formulations. The scientific literature is replete with evidence supporting the beneficial effects of many minerals, vitamins, antioxidants, anti-inflammatories, and growth factors in terms of skin hydration and firmness. Here, we describe and evaluate HPE formulation designed to improve skin appearance.

Non-FDA Approved Drug/Products

This study is testing non-FDA approved cosmetic products:

(1) HPE Skincare: Intensive Hydrating Elixir (active ingredient: HPE)

(2) Market-competitor: Skinceuticals C.E. Ferulic (active ingredients: Vitamin C, Vitamin E and ferulic acid serum).

IND application was not submitted to the FDA (cosmetic use, herbals, botanicals, supplement). These products are currently available over-the-counter for cosmetic use (www.hpeskincare.com).

HPE is an extracellular vesicle (EV) product manufactured by industry sponsor, RION (Rochester, MN). It represents a novel off-the-shelf regenerative cosmetic product for skin rejuvenation. HPE is a leukocyte depleted allogeneic product derived from human U.S. sourced pooled apheresed platelets (derived from screened healthy donors). HPE is comprised of platelet conditioned medium derived extracellular vesicles rich in anti-inflammatory and angiogenic growth factors. HPE is aseptically processed, pyrogen-free and does not contain preservatives. Sourced platelets are received by a compliant manufacturing facility from FDA registered/licensed blood banks, where they remain frozen until further processing. At that time platelets are thawed, lysed, filtered through a leukocyte reduction filter and undergoes staged sterile filtration to further eliminate cell, cell fragments and non-EV subcomponents prior. Following pooling and serial freeze-thaw cycles derived HPE EVs are lyophilized and packaged. The end-product consists of platelet extracellular vesicles rich in anti-inflammatory cues and antioxidant enzymes, is manufactured without addition of any excipients, compounds, preservatives, or biologically active molecules. This product has undergone human repeat insult patch testing on 100 subjects without any evidence of skin sensitization and has been tested in a collagen formulation, documenting anti-aging impact around the eyes in a 35-subject study.

HPE was developed with partnership from Vanicream (Pharmaceutical Specialties, Inc., Rochester, MN) and formulated without parabens, petrolatum, phthalates, and SLS. Plus, it's fragrance-free.

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INTENSIVE
HYDRATING
ELIXIR

BATCH NO. 001

HYDRATE + RESTORE

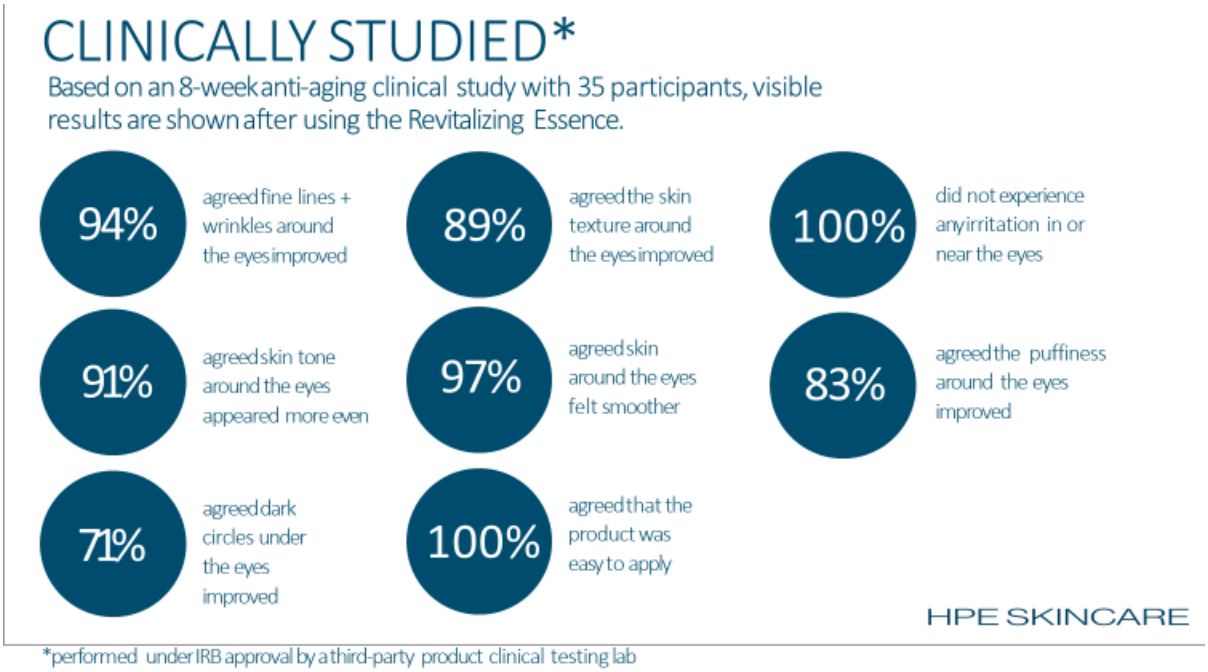
HPE SKINCARE

Usage: Before applying to face or targeted areas, use a gentle exfoliating cleanser. We recommend applying in the morning and night. Wait 10-15 minutes or until fully absorbed before layering with additional products.

Ingredients: Purified Water, Glycerin, Pentylene Glycol, Panthenol, Human Platelet Extract, Polyacrylate Crosspolymer-6, Hyaluronic Acid, Caprylyl Glycol, 1,2-Hexanediol, Arginine, Hydrolyzed Gelatin, Silanetriol

Warning: Use only as directed. For external use on the skin only. Do not apply to broken skin. Avoid direct contact with eyes. If contact occurs, rinse thoroughly with water. Discontinue use if skin irritation develops.

15 ml e / 0.5 fl.oz.



*performed under IRB approval by a third-party product clinical testing lab

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SKINCEUTICALS
ADVANCED SKINCARE BACKED BY SCIENCE

C E FERULIC® WITH 15% L-ASCORBIC ACID

A patented daytime vitamin C serum that delivers advanced environmental protection and improves the appearance of fine lines and wrinkles, loss of firmness, and brightens skin's complexion. Now clinically proven to reduce combined oxidative damage from free radicals generated by UV, Ozone, and Diesel Exhaust by up to 41%. U.S. Patent No. 7,179,841.

SKIN TYPES: Dry, Normal, Combination, Sensitive

SKIN CONCERN: Sensitized, Dehydrated, Discoloration, Aging

FEATURES:
C E Ferulic features a synergistic antioxidant combination of 15% pure vitamin C (L-ascorbic acid), 1% vitamin E (alpha tocopherol), and 0.5% ferulic acid to enhance protection against environmental damage caused by free radicals that can contribute to atmospheric aging. C E Ferulic is now proven to reduce combined oxidative damage generated by UVA/UVB rays, ozone pollution, and diesel engine exhaust by up to 41%. In addition to antioxidant protective benefits, C E Ferulic improves signs of aging and photodamage, the appearance of lines and wrinkles, and the loss of firmness, while brightening skin's complexion.

BENEFITS:
- Provides advanced environmental protection by neutralizing damaging free radicals
- Visible anti-aging benefits, such as the improvement of the appearance of lines and wrinkles, loss of firmness, and brightens skin's complexion
- Neutralizes free radicals on the upper layer of the skin to help prevent the impact of ozone damage to skin
- Once absorbed, this vitamin C serum remains effective for a minimum of 72 hours
- Paraben-free and ideal for normal, dry, and sensitive skin types
- Tested suitable for use post-laser, always consult with a physician for individual post-procedure care

- **15% L-ascorbic acid**
  Lauded for its superior antioxidant benefits, this highly potent form of pure vitamin C helps neutralize free radicals and protect against oxidative stress while providing visible anti-aging benefits.
- **1% alpha tocopherol**
  This pure form of vitamin E neutralizes free radicals and replenishes skin lipids. It has been proven to act synergistically with vitamin C.
- **0.5% ferulic acid**
  A plant-based antioxidant, ferulic acid neutralizes free radicals and enhances the antioxidant benefits and stability of vitamins C and E.

Learn more about L-ascorbic acid

Learn More About Ferulic Acid

See All Ingredients −

aqua / water / eau, ethoxydiglycol, ascorbic acid, glycerin, propylene glycol, laureth-23, phenoxyethanol, tocopherol, triethanolamine, ferulic acid, panthenol, sodium hyaluronate

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Study Design and Methods

Methods: *Describe in lay terms, completely detailing the research activities that will be conducted by Mayo Clinic staff under this protocol.*

80 participants over the age of 40 with moderate photoaging (dyschromic facial skin with fine lines and wrinkles) will be recruited from Mayo Clinic Center for Aesthetic Medicine and Surgery (CAMS) to participate in this study. After signing written informed consent, eligible subjects will be instructed to apply topical HPE to entire face and decolletage twice daily, in the morning and at bedtime. In addition, subjects will be instructed to apply topical HPE to the right dorsal hand and market-competitor group (Skinceuticals^{TM} C.E. Ferulic) to left dorsal hand. Select subjects (n=20) will also apply topical HPE to the right upper inner arm for skin biopsies at week 12. A baseline skin biopsy will be obtained from untreated skin on the left upper inner arm from the same subjects. Subject renumeration will be provided for skin biopsies.

Subjects will undergo clinical evaluations as outlined below on day 0, week 6 and week 12, which will include a subject questionnaire rating subjective parameters (Global Aesthetic Improvement Scale and Cosmetic Improvement Scale) and high resolution, standardized imaging (Canfield VISIA CR Gen 5 with 3DPrimos) to evaluate targeted fluorescence imaging of protoporphyrin-IX and coproporphyrin-III for measuring microbial activity, standard white light and polarized imaging for textural and topographical information, RBX® Red/Brown processing for measuring pigmentation, and 3D imaging with Primos for roughness and volumetric measurements. All subjects will have skin microbiome analysis performed from the face at baseline and week 12 unless they did not meet criteria (i.e., washed face 3-hours prior to clinical visit, which would shift the skin microbiome; or prior allergy to adhesives) or unless they decline. Skin microbiome analysis would be performed by skin swab and tape-stripping. Subjects (up to n=80) who are interested in being involved for a longer duration in the study will be followed to 26-week and 52-week time points for long-term analysis of topical HPE on facial and decolletage skin per subject preference. Subject renumeration will be provided for skin biopsies, and follow-up 26- and 52-week visits.

At the end of the study participants will be asked if they would like to participate in a video interview using an interview guide. Participants that don't continue use of the products after 12 weeks, will be given the option to return to the clinic for photos at 6 months and 12 months. These participants will be given another bottle of the study product.

We will use a zoom type platform and capture study participants thoughts and attitudes towards overall skincare routines and HPE (plated)^{TM} SkinScience product. Each selected patient will be recorded in a 30-minute interview. The interviews will be transcribed, edited for patient testimonials (2–3-minute videos) and used in marketing and patient education materials.

Amendment 2X concentration study:

We will enroll an additional 50 patients as part of a substudy. We aim for 30-40% people of color. We will partner with DMC BIPOC entrepreneur (MotivateMe) for community outreach and engagement.
They will have the same study visits and surveys at baseline, 6 weeks, and 12 weeks. Biopsies will be optional and can be done on the left forearm and/or upper inner arm at baseline and at 12 weeks. At 12 weeks the biopsy

will be on the right arm in a similar area as the left. Participants will receive $250 for each biopsy.All subjects will have microbiome (swab and tape) at baseline and 12 weeks. All subjects will have standard face/neck photography and 3D imaging at all three visits. Subjects in this 2X study will be reimbursed $50 for the first two study visits. They will receive $100 for the 12 week visits. Same inclusion and exclusion criteria. We will ask males to be shaved for their photos.

Participants will be given the option to return to the clinic for photos at 6 months and 12 months. These participants will be given another bottle of the study product.

We will pursue collaboration with MotivateMe, LLC local Latinx/Hispanic entrepreneur for skin of color patient recruitment. This is a collaborative effort with Center for Health Equity and Community Engagement Research

We have been approached by the Rochester Post Bulletin for an article regarding our clinical study and skin of color patient recruitment. This has been vetted by Mayo Pubic Affairs team, but there is concern that IRB may consider this article as a recruitment tool. We will also use social media (see social media plan attachment)

Subject Information

Target accrual is the proposed total number of subjects to be included in this study at Mayo Clinic. A "Subject" may include medical records, images, or specimens generated at Mayo Clinic and/or received from external sources.

Target accrual: 80 + 50 from substudy = 130

Subject population (children, adults, groups):

Adults age 40 to 85 years with moderate photoaging.

Inclusion Criteria:

1. Adult males and females ages 40 to 85 years
2. Persons of childbearing potential must have a negative pregnancy test prior to receiving the study product and will agree to use adequate contraception (hormonal or barrier method or abstinence) from the time of screening to a period of 2 years until discontinuation of treatment. Females of childbearing potential are defined as premenopausal and not surgically sterilized, or post-menopausal for fewer than 2 years. A urine pregnancy test will be performed prior to the administration of the study product to confirm negative results. If the urine pregnancy test is positive, the study product will not be administered, and the result will be confirmed by a serum pregnancy test. Urine pregnancy tests will be performed by qualified personnel using kit. Persons becoming pregnant during the study will continue to be monitored for the duration of the study or completion of the pregnancy, whichever is longer. Monitoring will include perinatal and neonatal outcome. Any SAEs associated with pregnancy will be recorded.
3. All skin phototypes ≥ grade I of Fitzpatrick's classification.

4. Mild-to-moderate global face wrinkles and mild-to-moderate global fine lines based on a modified Griffiths' 10-point scale [3].
5. Fully understanding of the requirements of the study and willingness to comply with the treatment plan, including laboratory tests, diagnostic imaging, and follow-up visits and assessments.
6. Volunteer willingness to discontinue any other anti-aging topical or parenteral treatments for the duration of the study.
7. Can provide written informed consent and complete HIPAA documentation after the nature of the study is fully explained and prior to any study-related treatment.
8. Can provide written informed consent to being photographed for purposes of treatment for medical, scientific purposes.

Exclusion Criteria:

1. Pregnant or nursing, or planning on becoming pregnant during the study period
2. Subjects who have had an antiaging or aesthetic treatment prior to the study: Botox or Botox-like products, peelings, plastic surgery, resurfacing with Laser, IPL, threats, radiofrequency treatments, hyaluronic acid treatment, Plasma-Rich Platelets treatment, or any other specific treatments prone to change the skin aspect during the last 6 months
3. Individuals with a history of any dermatological disease or condition, including but not limited to active atopic dermatitis, psoriasis, eczema, active seasonal allergies, collagen diseases, or skin cancer involving the treated sites within the past 6 months
4. Cutaneous marks on the experimental area which could interfere with the assessment of skin reactions (pigmentation problems, scar elements, over-developed pilosity, ephelides, and nevi in too great quantity, sunburn, beauty spots, freckles, etc.)
5. Participants with asymmetric photodamage on dorsal hands due to environmental exposures (i.e., golfing) and/or other skin lesions including burns or scars resulting in significant skin surface variability between dorsal hands
6. Eczematous reaction still visible, scar, or pigmentary sequelae of previous tests on the experimental area
7. Allergy to colophony or nickel.
8. Allergy or reactivity to drugs, food or cosmetic products previously observed, including perfumes or cologne products.
9. Skin hyper-reactivity.
10. Forecast of intensive sun, tanning bed use or UV phototherapy during the test period.
11. Treatment with prescription-strength Vitamin A acid or its derivatives within 3 months before the beginning of the study.
12. Treatment with topical steroids on the experimental area within 16 days before the study.

Study constraints:

1. No application of products on the experimental area (except the suggested ones), particularly any anti-aging cosmetic products except for sunscreen.
2. No change in hygiene habits
3. No application of any cosmetic moisturizing products on the face or any makeup on face and lips, on the day of study evaluations
4. No change in the way of life or in the physical activity

5. No change in dietary activities, or any treatment that significantly impacts body weight
6. No exfoliating treatment on the experimental areas
7. Description of any treatment undertaken during the study and all eventual deviations

Review of medical records, images, specimens

Check all that apply (data includes medical records, images, specimens).

☐ Only data that exists before the IRB submission date will be collected.

Date Range for Specimens and/or Review of Medical Records:
Examples: *01/01/1999 through 12/31/2015*, or all records through *mm/dd/yyyy*.

Note: The Date Range must include the period for collection of baseline data, as well as follow-up data, if applicable.

☒ The study involves data that exist at the time of IRB submission **and** data that will be generated after IRB submission. Include this activity in the Methods section.
Examples

- The study plans to conduct a retrospective chart review and ask subjects to complete a questionnaire.
- The study plans to include subjects previously diagnosed with a specific disease and add newly diagnosed subjects in the future.

☐ The study will use data that have been collected under another IRB protocol. Include in the Methods section and enter the IRB number from which the research material will be obtained. *When appropriate, note when subjects have provided consent for future use of their data and/or specimens as described in this protocol*.

Enter one IRB number per line, add more lines as needed

☐ Data ☐ Specimens ☐ Data & Specimens _____

☐ Data ☐ Specimens ☐ Data & Specimens _____

☐ Data ☐ Specimens ☐ Data & Specimens _____

Data Analysis

Power analyses may not be appropriate if this is a feasibility or pilot study, but end-point analysis plans are always appropriate even if only exploratory. Provide all information requested below, or provide justification if not including all of the information.

Data Analysis Plan:

All continuous data will be analyzed using independent 2-group t tests and reported as means ± standard deviations. Categorical data will be compared between the 2 groups using chi-square tests and reported as counts and percentages. Nonparametric equivalents Wilcoxon rank-sum and Fisher exact tests will be used as needed for nonnormal distributions and low variable numbers, respectively. Treatment effects on outcomes will be compared between baseline versus HPE and HPE versus market competitor group with a significance level of $p < 0.05$.

Endpoints

Primary:
Evaluation of the tolerability of the study product.

a. Nature, incidence, and severity of adverse events (AEs)
    1) Defined as any untoward or undesirable medical occurrence in the form of signs, symptoms, abnormal findings, or diseases that emerge or worsen relative to baseline (i.e., if present upon study entry) during the study regardless of causal relationship.
    2) Assessment
        i. Incidence and severity of AEs (mild, moderate, severe)
        ii. Relationship of AEs to study product (probable, possible, unlikely, unrelated)
            1. If possibly or probably related to the study product, determine whether related to the product itself or procedures pertaining to delivery of the study product.
        iii. Incidence of serious adverse events (SAEs) - defined as AEs that result in death, life threatening adverse experiences, hospitalization, new or prolonged disability/incapacity, persistent or significant congenital defect/anomaly, or other events that in the opinion of the PI may have adversely affected the rights, safety, or welfare of the subjects or others, or substantially compromised the research data.
    3) Methods
        i. Spontaneous subject reports
        ii. Subject interview by study personnel
        iii. Clinical examination during face-to-face clinic follow-ups
    4) Timing
        i. Baseline
        ii. 6-week post-treatment (acute)
        iii. 12, 26- and 52-weeks post-treatment (delayed)

Change in photoaging scores from Baseline at 6,12, 26- and 52-weeks.

a. Canfield VISIA CR Gen 5 with 3DPrimos will be used to evaluate qualitatively and quantitatively skin profile changes (color evenness and roughness measurements), and to assess wrinkle count, depth, and volume by a full-aligned facial and bilateral dorsal hands images.

Information about Canfield VISIA CR is a high-resolution clinical imaging system (21 Megapixel resolution 45MP Canon EOS R5) which uses LED lighting. No radiation-based exposure. It captures high-resolution

photographic images. More information on prior model (without 3D Primos):
https://www.canfieldsci.com/common/docs/products/1/brochures/VISIACRBrochure.pdf

Secondary:

1) Dermatopathologic evaluation of the skin
   a) Skin microbiome: To define skin facial microbial clades, a standard swab and tape stripping method will be used on all interested subjects. Both methods are minimally invasive and can yield optimal results. All subjects will be offered skin microbiome analysis unless they did not meet criteria (i.e., washed face 3-hours prior to clinical visit, which would shift the skin microbiome; or prior allergy to adhesives) or unless they decline
      i) Skin microbiome: Skin swab collection (n=80)
      Skin bacteria will be collected by the swabbing method as described in previous studies [4]. In brief, the left and right malar cheeks will be gently swabbed with a single dry cotton swab in a Z-stroke manner [5]. For skin microbiome, the swab head will be stored in a sterile 1.5-mL centrifugation tube at −80°C until ready for analysis.
      ii) Skin microbiome: Tape-stripping collection (n=80)
      Collection of skin bacteria by the tape-stripping method is based on the method described in previous studies [6]. Two D-squame standard sampling discs (Monaderm, Monaco, France) will be applied consecutively to the skin area (one tape on the left malar cheek, one tape on the right malar cheek), using a new piece for each application, for 1 minute. The tape will be peeled off from skin with sterile forceps. For skin microbiome, the two tapes will be pooled for analysis and stored in a sterile 1.5-mL centrifugation tube at −80°C until ready for analysis.

   b) Skin biopsies: To define histological changes to collagen, elastin, and cellular senescence, 5-mm skin punch biopsies will be obtained. Subjects (n=30) will be instructed to apply topical HPE (right upper inner arm). At baseline, a 5-mm punch biopsy from the left upper inner arm will be obtained from untreated skin. At week 12, a 5-mm punch biopsy from the right upper inner arm will be obtained from HPE-treated skin. Each subject (n=30) will therefore undergo 2 total skin biopsies. Subject renumeration provided. The first 30 willing subjects will be recruited.

i) In this study, we will remove a small piece of skin from the left upper inner arm (untreated) at baseline and right upper inner arm at week 12 (treated - topical HPE). This is called a skin biopsy.
ii) The site(s) or area of the skin identified for skin biopsy will be cleaned with antiseptic and may be numbed by injecting a small amount of numbing medicine, through a small needle. Some people (fewer than 1 in 10,000) are allergic to the shot you will get to numb the area where the skin is taken. The biopsy procedure is conducted by taking a larger circular tool or needle (about 1/5th of an inch in diameter), inserting it into the skin and removing the core piece that fits inside the circle. There could be bleeding from the site, and this might require placement of a stitch. Heavy bleeding from a skin biopsy is rare. Other complications could include pain, and there is the possibility of the biopsy site becoming infected. Skin biopsies cause infections about 10% of the time. A small scar will form at the biopsy site. The scar is usually much smaller than the original biopsy. The procedure is conducted using sterile instruments after disinfectant or antiseptic cleaning of the skin.

2) Self-assessment [Time Frame: 6, 12, 26- and 52-weeks]
   a) Global Aesthetic Improvement Scale [7]:
      i) 1 Exception: Very much improved and optimal cosmetic result for the subject
      ii) 2 Moderate: Much improved and marked improvement in condition, but not optimal for subject; touch-up would slightly improve the result
      iii) 3 Slight: Improved with obvious improvement but touch-up or retreatment indicated
      iv) 4 No change: Appearance essentially the same as original condition
      v) 5 Worse: Appearance worse than original condition
   b) Cosmetic Improvement Scale [8]: The following parameters will be scored as a percentage of total responses from participants.
      i. Percentage of subjects satisfied or very satisfied with the treatment
      ii. Percentage of subjects who would recommend the treatment to a friend
      iii. Percentage of subjects reporting high likelihood of continuing treatment
      iv. Percentage of subjects reporting treatment positively or negatively affected self-esteem
      v. Percentage of subjects reporting no, minimal, moderate, or significant improvement in fine lines and wrinkles, skin tone, skin smoothness and/or puffiness

Data collection

Data collection, processing, transmission, and storage will follow Mayo Clinic Data Protection Standards. Redcap, a Mayo-endorsed, password protected HIPAA-compliant system, will be used as the electronic data collection and management system. It allows data tracking and downloads to statistical analysis packages. Participant informed consents will be tracked using Ptrax. Participant questionnaires will be collected using REDCap.

Canfield VISIA CR Gen 5 with 3DPrimos will be used to evaluate qualitatively and quantitatively skin profile changes. If Canfield is processing any data on behalf of Sponsor, Canfield shall do so in strict accordance and compliance with applicable laws, rules, and regulations, including to the extent applicable, the Health Insurance Portability and Accountability Act of 1996 and the Regulation (EU) 2016/679 of the European Parliament and the Council of 27 April 2016 on the protection of natural persons with regard to the processing of personal data and on the free movement of such data (GDPR), and with Sponsor's instructions, and it shall not utilize said data for any other purpose whatsoever without obtaining data subject's consent first.

Additionally, Canfield shall take all appropriate technical and organizational measures which are necessary to prevent the unauthorized or unlawful processing of, the unauthorized or unlawful disclosure of and/or the accidental loss or destruction of, or damage to, such data, including without limitation, implementation and enforcement of administrative, technical and physical security policies and procedures. Incident Reporting. Canfield shall promptly notify Sponsor in the event of a security breach or incident involving any data which Canfield is processing on behalf of Sponsor.

Study procedures

The skin biopsies, skin swabs and long-term follow-up visits will be obtained separately from 80 subjects.

- n=80 subjects
- Entire face and decolletage HPE (n=80)
- HPE (n=80) to be applied on the right dorsal hand
- Skinceuticals CE Ferulic (n=80) to be applied on the left dorsal hand
- Skin biopsy (baseline): Left upper inner arm – untreated (n=30)
- Skin biopsy (week 12): Right upper inner arm – HPE treated (n=30, same subjects as above)
- Microbiome analysis (baseline): Left and right malar cheek – untreated (n=80 with both skin swab and tape-stripping method)
- Microbiome analysis (week 12): Left and right malar cheek – HPE treated (n=80 with both skin swab and tape-stripping method)

N=30 - Skin biopsy: Two total skin biopsies will be performed per subject (n=30 subjects). In this study, we will remove a small piece of skin from untreated skin on the left upper inner arm (baseline) and HPE treated area on the right upper inner arm (week 12). This is called a skin biopsy.

The site(s) or area of the skin identified for skin biopsy will be cleaned with antiseptic and may be numbed by injecting a small amount of numbing medicine, through a small needle. Some people (fewer than 1 in 10,000) are allergic to the shot you will get to numb the area where the skin is taken. The biopsy procedure is conducted by taking a larger circular tool or needle (about 1/5th of an inch in diameter), inserting it into the skin and removing the core piece that fits inside the circle. There could be bleeding from the site, and this might require placement of a stitch. Heavy bleeding from a skin biopsy is rare. Other complications could include pain, and there is the possibility of the biopsy site becoming infected. Skin biopsies cause infections about 10% of the time. A small scar will form at the biopsy site. The scar is usually much smaller than the original biopsy. The procedure is conducted using sterile instruments after disinfectant or antiseptic cleaning of the skin.

N=80 - Skin swab or Tape-stripping: Method validation will be performed at baseline visit. All subjects will be evaluated by skin swab method and tape stripping method at baseline visit. All 80 subjects will receive the skin swab method and tape stripping method at week 12 visit for microbiome analysis post-HPE treatment. All subjects will be offered skin microbiome analysis unless they did not meet criteria (i.e., washed face 3-hours prior to clinical visit, which would shift the skin microbiome; or prior allergy to adhesives) or unless they decline

In this study, bacteria will be collected by either of two methods (standard swab and tape stripping method) from your skin to culture the bacteria that normally live on the skin.
Standard swab: we will gently rub a Q-tip like swab on your skin around 4-5 times to obtain the sample.

Tape-stripping: we will use 2 specialized, transparent, adhesive disks, maintained on the skin for 1 minute to obtain the samples. You will be instructed to avoid contact with water and soap at least 3 hours prior to sample collection and refrain from wearing make-up on the day of sample collection.

Video Interview

Participants will also be asked if they would like to be part of a video interview to talk about their experience in the study and with the products.

Screening Visit / Baseline Visit (Visit 0)

The screening visit will take place prior to enrollment to determine if the potential subject is eligible to participate in the study.

Informed consent must be obtained with signing of the ICF prior to performing any study-related procedure.

Assessments during the screening visit will include:

- Demographic data
- Medical and surgical history
- Medication history
- Concomitant medication
- Concomitant procedures
- Physical examination

Based on the result of the screening visit and review of the eligibility criteria, the PI will assess each potential subject's eligibility for study inclusion. If the subject is found eligible, she can then be enrolled. The screening visit results will be recorded in the subject's electronic case report form (e-CRF) and the subject will be scheduled for a subsequent visit to complete additional baseline assessments,

Enrolled subjects will then initiate baseline visit (V0) at the same time during which they will complete their baseline assessments and initiate their treatment.

General assessment

- Baseline VISIA-CR imaging
- Photography
- Skin microbiome facial swab and tape-stripping (n=80 skin swab and tape-stripping)
- Skin biopsy (n=30)
    - Left upper inner arm (untreated skin)

Treatment

- Treatment: Topical HPE twice daily for study duration.
- Study staff will demonstrate treatment application.
- Subject instructions and treatment log will be provided.
- Each subject will receive the first cosmetic kit, which will include gentle cleanser, unscented bar soap, HPE™ product, Skinceuticals™ CE Ferulic serum, sunscreen and moisturizer. A second cosmetic kit will be provided at week 6 visit.

- For questions, subjects will be provided contact email: aesthetic.research@mayo.edu

Visit 1: Follow-up at 6-weeks

- VISIA-CR imaging
- Photography
- Topical treatment jars will be weighed to ensure correct application amount.
- Subject treatment log will be reviewed.

Specific assessment (cosmetic evaluation questionnaires)
- Global Aesthetic Improvement Scale
- Cosmetic Improvement Scale

Treatment assessment
- Continuation of Topical HPE twice daily as indicated.
- Study staff will re-demonstrate treatment application.
- Each subject will receive their second cosmetic kit, which will include gentle cleanser, unscented bar soap, HPE™ product, Skinceuticals™ CE Ferulic serum, sunscreen and moisturizer.
- For questions, subjects will be provided contact email: aesthetic.research@mayo.edu

Visit 2: Follow-up at 12-weeks

- VISIA-CR imaging
- Photography
- Skin microbiome facial swab and tape-stripping (n=80 skin swab and tape-stripping)
- Skin biopsy (n=30)
    - Right upper inner arm (HPE)
- Topical treatment jars will be weighed to ensure correct application amount.
- Subject treatment log will be reviewed.

Specific assessment (cosmetic evaluation questionnaires)
- Global Aesthetic Improvement Scale
- Cosmetic Improvement Scale

Treatment assessment
Continuation of topical HPE twice daily on the face and decolletage only as indicated for 20 subjects.
- Study staff will re-demonstrate treatment application.
- Each subject will receive their second cosmetic kit, which will include gentle cleanser, unscented bar soap, HPE™ product, Skinceuticals™ CE Ferulic serum, sunscreen and moisturizer.
- For questions, subjects will be provided contact email: aesthetic.research@mayo.edu

Visit 3 and 4: Long-term follow-up at 26- and 52-weeks. Cohort 2 (n= up to 80)

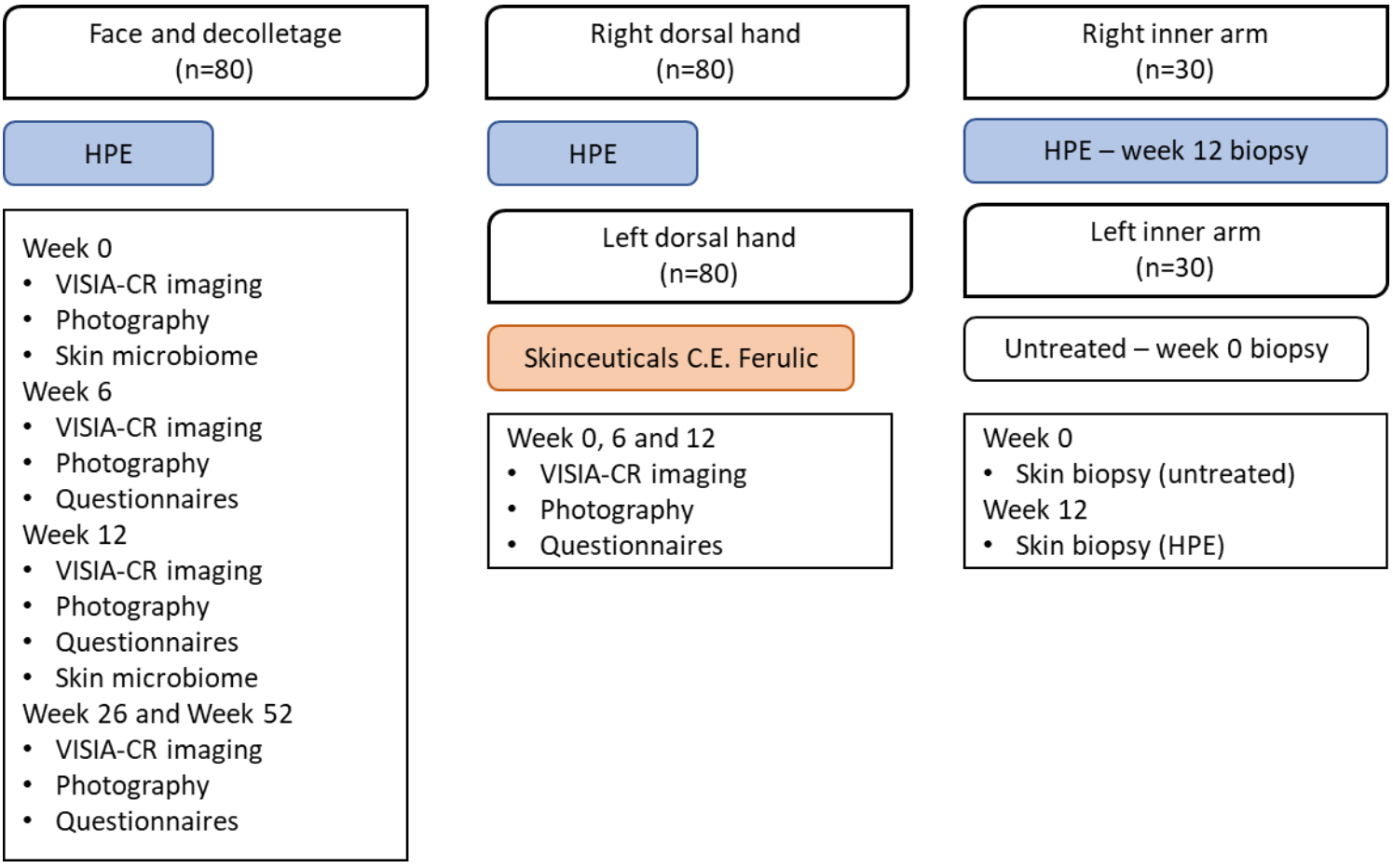
- VISIA-CR imaging

- Photography
- Topical treatment jars will be weighed to ensure correct application amount.
- Subject treatment log will be reviewed.

Specific assessment (cosmetic evaluation questionnaires)

- Global Aesthetic Improvement Scale
- Cosmetic Improvement Scale

Figure 1: Study Summary



MAYO CLINIC

Table 1: Summary of Monitoring and Follow-Up Assessments

Assessment	Screening / Baseline Visit (Visit 0)	6 Weeks (Visit 1) (±4 Days)	12 Weeks (Visit 2) (±7 Days)	26 Weeks* (6 months) (Visit 3) (±14 Days)	52 Weeks* (12 months) (Visit 4) (±30 Days)
Informed Consent and HIPAA Authorization	X	—	—	—	—
Questionnaires and Assessments	All Encounters				
Skin biopsy*	X	—	X	—	—
Microbiome analysis (skin swab and tape-stripping)	X	—	X	—	—
VISIA-CR imaging	X	X	X	X	X
Photography	X	X	X	X	X
AE Evaluation	All Encounters				
Medical History	X	—	—	—	—

* Cohort (skin biopsy) (n=30) – Baseline visit will include a single biopsy (left upper inner arm) from untreated skin. 12-week visit will include a single biopsy from HPE-treated (right upper inner arm). Long-term participation at 26 weeks and 52 weeks will be offered to all subjects if interested.

References

1. Kammeyer, A. and R.M. Luiten, *Oxidation events and skin aging.* Ageing Res Rev, 2015. **21**: p. 16-29.
2. Pinnell, S.R., *Cutaneous photodamage, oxidative stress, and topical antioxidant protection.* J Am Acad Dermatol, 2003. **48**(1): p. 1-19; quiz 20-2.
3. Nguyen, T.Q., et al., *A Randomized, Double-blind, Placebo-controlled Clinical Study Investigating the Efficacy and Tolerability of a Peptide Serum Targeting Expression Lines.* J Clin Aesthet Dermatol, 2021. **14**(5): p. 14-21.
4. Ogai, K., et al., *A Comparison of Techniques for Collecting Skin Microbiome Samples: Swabbing Versus Tape-Stripping.* Frontiers in Microbiology, 2018. **9**(2362).
5. Rushing, J., *Obtaining a wound culture specimen.* Nursing, 2007. **37**(11): p. 18.
6. Sølberg, J., et al., *The stratum corneum transcriptome in atopic dermatitis can be assessed by tape stripping.* J Dermatol Sci, 2021. **101**(1): p. 14-21.
7. Muti, G.F., *Midface 3D restoration with an innovative high G' filler.* J Cosmet Dermatol, 2021. **20 Suppl 2**: p. 7-11.
8. Pompilus, F., et al., *Development and validation of a novel patient-reported treatment satisfaction measure for hyperfunctional facial lines: facial line satisfaction questionnaire.* J Cosmet Dermatol, 2015. **14**(4): p. 274-85.