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# Ovolux™ Brand Eggshell Membrane Reduces the Clinical Signs of Aging by Improving Skin, Hair & Fingernail Appearance, Texture, and Biomechanical Properties: A Single Center, Randomized, Double Blind, Placebo Controlled Clinical Trial

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## Abstract

**Background:** As the population of people aged 65 and older increases, greater attention is being paid to issues surrounding skin aging. This study was conducted to evaluate the efficacy of an oral unhydrolyzed eggshell membrane (ESM) ingredient (branded Ovolux™) versus placebo on both objective and subjective markers of skin aging in middle-aged and senior adults, and also to assess the possible benefits of Ovolux™ on the health of hair and fingernails. **Methods:** Sixty-three healthy, men and women (aged 35 - 66) were randomly assigned to receive either oral ESM 300 mg (n = 32) or placebo (n = 31) once daily for 12 weeks. The primary endpoint was any statistically significant improvement in skin elasticity in the anterior region of the temporal fossa of the face versus placebo evaluated at 12 weeks of treatment based upon measurements taken with the Cutometer® MPA 580 multi-probe system. Secondary endpoints were improvement in skin firmness, viscoelasticity, hydration, or trans-epidermal water loss versus placebo. Additional secondary endpoints were any improvement in skin, hair, or fingernail subjective parameters versus placebo as determined via Patient's Assessments. **Results:** Supplementation with Ovolux™ ESM produced a significant absolute treatment effect ($TE_{abs}$) versus placebo after both six weeks ($TE_{abs}$ +8.4%, p = 0.019) and twelve weeks of treatment ($TE_{abs}$ +12.2%, p = 0.002) for the primary outcome measure total elasticity (R2). There were also significant treatment responses versus placebo for the objective measures of firmness (R0), net elasticity (R5),

and viscoelasticity (R6) at both timepoints (R0: Week 6, −23.9%; Week 12, −16.8%) (R5: Week 6, +9.5%; Week 12, +10.6%) (R6: Week 6, −17.9%; Week 12, −17.7%), as well as for skin hydration versus placebo at both 6 weeks (+8.3%) and 12 weeks (+8.6%) of treatment. There were also significant improvements in the ESM treatment group versus placebo in the subjective measures of skin appearance, skin texture, overall skin health, hair texture, and overall hair health as determined by patient assessments. **Conclusions:** Ovolux™ provided significant broad-based improvements in critical biomechanical skin properties versus placebo, including elasticity, firmness, and viscoelasticity. These effects, along with improvement in skin hydration, were observed as early as 6 weeks and continued through the end of the 12-week study. Treatment with ESM was reported to be well-tolerated. Ovolux™ ESM appears to be a natural solution to help skin to resist the epidermal thickening, the dermal thinning, and the skin laxity and elastosis found in intrinsically aging skin. The Clinical Trial Registration number is: NCT06148337.

## Keywords

Eggshell Membrane, Skin Aging, Elastosis, Laxity

## 1. Introduction

Globally, the number of individuals over age 65 is the fastest growing population, with 1 in 10 persons worldwide being in this group as of 2021 [1]. But with this growth in the aging population, comes an associated increase in health-related effects of the aging process. Aging impacts virtually all processes and systems within the human body, the most outwardly visible of which is frequently the skin. Skin aging occurs as a direct result of the combined effects of time (intrinsic aging) and the environment (extrinsic aging). The degree of skin aging over time is directly linked to non-modifiable (intrinsic) factors like age, gender, anatomical location, and genetics that collectively influence the chronological age of skin [2]. However, skin aging is also linked to exposure to damaging, but modifiable, environmental (extrinsic) risk factors such as UV radiation, chemical air pollutants, and nutritional deficiencies [2]. Chronic disease conditions often manifest in the appearance of the skin [3] [4]; therefore, attention to the skin as well as intimately associated tissues such as hair and fingernails is not just one of cosmetic interest, but also a general health necessity. As a result, there has been a growing trend toward addressing the nutritional deficiencies occurring within the body that are manifest in the skin, hair, and fingernails. Appropriate supplementation can help ensure that key skin-supportive nutrients are available to help compensate for those that decrease with chronological aging or are stressed or damaged because of environmental challenges.

Skin is the largest organ in the human body and serves a number of vital functions, including serving as a barrier to environmental insult (physical, chemical,

or biological), preventing excess water loss from the body, and helping to regulate body temperature. Skin is composed of three main layers: the epidermis, the dermis, and subcutaneous tissue. The outermost layer, the epidermis, is a stratified squamous epithelium layer that is primarily comprised of keratinocytes. The epidermis is a continually renewing layer and gives rise to derivative structures such as hair follicles, fingernails, and sweat glands [5]. The intermediate layer, the dermis, is fundamentally made up of the fibrillar structural protein type I collagen and, to a lesser extent, the fibrillar elastic protein elastin [5]. The extracellular matrix (ECM) of the dermis is additionally composed of water-binding glycosaminoglycans (GAGs), in the form of proteoglycans, including hyaluronic acid (HA), dermatan sulfate (DS), and heparan sulfate (HS) [6]. These GAGs help to keep normal skin plump, soft and hydrated. The viscoelastic properties of the dermis protect the skin from mechanical stress providing pliability, elasticity, and tensile strength. The dermis provides structural support for the epidermis, blood vessels, hair follicles, and fingernails. Because of this, the structural integrity of the dermis is vital for the normal functioning of skin. Below the dermis lies the subcutaneous tissue which contains small lobes of fat-producing cells (lipocytes).

In intrinsically (chronologically) aging skin, the dermis becomes 20% thinner [7] due to decreasing levels of collagen [8], which leads to fine wrinkling, fragility, and laxity (sagging) [9]. The overall collagen content per unit area of skin surface declines by approximately 1% per year in aging skin. Total GAG levels are also substantially decreased in intrinsically aging skin in both the epidermis and the dermis, while HA is mainly reduced in the epidermis and HS is mainly reduced in the dermis [6]. In extrinsically aging skin, there is epidermal thickening with the dermis producing markedly decreased levels of fragmented and disordered collagen fibrils that are increasingly replaced by abnormal elastin [8]. Total GAGs, and particularly HA, are substantially reduced in extrinsically aging skin [7]. Combined, this leads to skin dryness with deep furrows, irregular pigmentation, sallowness, laxity, elastosis, and a leathery appearance [9].

Eggshell membrane (ESM) is the bi-layered fibrous membrane of the chicken egg separating the hard outer shell from the liquid albumen (egg white), that naturally contains multiple nutrients that have been shown to be beneficial for skin care. The fibrous matrix of the membrane is primarily protein and contains collagen, predominantly type I, with smaller amounts of types V and X [10] [11]. Nearly 500 proteins have been identified in ESM via proteomics [12] [13], including six additional types of collagen (III, IV, VII, VIII, XII, and XXII) [13]. In addition to the proteins, ESM contains calcium and GAGs, including chondroitin sulfate (CS) [14], DS [15], keratan sulfate (KS) [14] [15], HS [14] and HA. Natural unhydrolyzed ESM and its content of collagens, elastin [10], and GAGs closely resembles the ECM constituents of human epidermis and dermis. As such, it has begun to be investigated for skin care. Oral supplementation of ESM was shown to upregulate ECM growth factors and reduce skin thinning in a mouse model of nutrient-deficient skin aging [16]. In a skin photoaging mouse model, ESM was

found to improve skin hydration, reduce trans-epidermal water loss (TEWL), and decrease wrinkle depth at a dose of 30 mg/kg for 12 weeks [17]. An open-label pilot clinical study in which 10 subjects consumed 300 mg of ESM for 5 weeks showed an 11% improvement in skin elasticity when compared to baseline [18]. Another pilot clinical study, conducted as a randomized controlled trial (RCT), in which 20 subjects consumed 900 mg of ESM or placebo twice daily for 8 weeks similarly demonstrated an improvement in skin elasticity versus placebo [19]. Additionally, an RCT in which 50 subjects consumed 300 mg of ESM for 8 weeks found an 18.3% net reduction in viscoelasticity (R6) versus placebo [20]. As such, ESM has shown promise to resist two of the major damaging effects (laxity and elastosis) of intrinsically aged skin.

Owing to the promise of this prior research of ESM as a "beauty from within" oral supplement, we set out to conduct an RCT with this material to evaluate a broader set of hair, skin, and fingernail parameters in a larger study population with a longer study duration.

## 2. Patients and Methods

### 2.1. Study Design

This study was conducted according to a single center, randomized, double-blind, placebo-controlled design in accordance with the U. S. Food & Drug Administration's principles of Good Clinical Practice (Title 21, Code of Federal Regulations, Parts 50 & 56 and ICH E6) and the Declaration of Helsinki (1996 version). The study protocol was approved by an independent, registered institutional review board (IRB), IRB Solutions, and subjects provided their written informed consent in order to participate. After meeting all inclusion/exclusion criteria at screening, eligible subjects were then randomized to receive either ESM powder or placebo in the order in which they were enrolled in the study using a permuted block randomization table consisting of four subjects per block. Treatment consisted once daily orally of either 300 mg of ESM powder or placebo. Treatment compliance was checked at clinic visits by participant interview and by counting the number of unused doses of the study capsules. All participants, clinical staff, and study management staff remained blinded to treatment assignment throughout the study.

Subjects completed skin, hair, and fingernail 10-point ordinal questionnaires at baseline and after 6 weeks and 12 weeks of treatment to evaluate their subjective responses to treatment effects for skin (appearance, texture, and overall health), hair (thickness, texture, overall health, and growth rate), and fingernails (appearance, strength, and growth rate). Following a period of 10 - 30 minutes at the study facility for acclimation to the study center environment, skin hydration was measured with a Corneometer® CM825 probe, TEWL was measured with a Tewameter® TM300 probe, and elasticity, firmness, and visco-elasticity were measured with a Cutometer® probe, all attached to a Courage + Khazaka (Cologne, Germany) Cutometer® MPA 580 with CTplus control software. Three replicate measurements

for each parameter were taken of the skin of the anterior region of the temporal fossa of the face (the crow's feet area). The Corneometer® CM825 probe assesses skin surface hydration by electrical capacitance using the stratum corneum as a dielectric membrane. Measurements are arbitrarily expressed as indices of hydration (0 - 99), which increase with increasing skin hydration. A score less than 30 implies very dry skin; a score of 30 - 60 indicates dry skin; and a score over 60 implies sufficiently moisturized skin (C + K Technical Guide). Moisture is constantly evaporating from the skin, however as the barrier function of the skin declines this evaporation rate increases. The Tewameter® TM300 probe continuously assesses the density gradient of the water evaporating from the skin indirectly via two pairs of sensors (temperature and relative humidity) inside a dual-open-ended hollow cylinder. Measurements were obtained over 25 seconds (s) and are expressed as evaporation rate in $g/m^2/h$, which decrease with decreasing TEWL. With healthy skin, a TEWL between 5 and 15 $g/m^2/h$ can be expected for room conditions of 20 - 22˚C and 40 - 60% relative humidity (RH) (C + K Technical Guide). The Cutometer® probe assesses skin firmness, elasticity, and viscoelasticity based on the well-established suction method that uses suction to draw the skin into the aperture of a probe. The resistance of the skin to deformation by a constant negative pressure (firmness) and its ability to return to its original position (elasticity/viscoelasticity) were evaluated. Measurements of the biomechanical properties of skin were performed with a 4 mm diameter probe according to the manufacturer's instructions with the following instrument settings: Mode 1, 450 mbar, on-time: 3 s, off-time: 3 s. The R0 (or Uf) parameter measures the maximum amplitude of skin deformation in mm and represents the passive behavior of the skin to force (firmness). Greater skin firmness results in a decrease in the R0 value. The R2 (or Ua/Uf) parameter measures the ratio of the complete (elastic + viscous) recovery of the skin to its original shape to the maximal skin deformation (total elasticity). Greater total skin elasticity results in an increase in the R2 value. The R5 (or Ur/Ue) parameter measures the ratio of the elastic recovery of skin to the elastic deformation of the skin (net elasticity). Greater net skin elasticity results in an increase in the R5 value. The R6 (or Uv/Ue) parameter measures the ratio of the viscoelastic skin deformation to the elastic skin deformation (viscoelasticity). Greater skin viscoelasticity results in a decrease in the R6 value.

## 2.2. Patients

All men and women, 35 - 70 years of age, who were generally healthy and free from any chronic skin conditions were considered for enrollment in the study. Subjects were required to discontinue all current prescription or over-the-counter (OTC) topical or oral medications expected to improve the health of skin, hair, or fingernails for the duration of the study (e.g. tretinoin, isotretinoin, hyaluronic acid, collagen, topical steroids, oral or topical antibiotics, topical retinol, topical benzoyl peroxide, topical glycolic acid, topical salicylic acid, etc.) in order to

participate. Subjects that were currently using skin, hair, or fingernail medications were eligible to participate in the study following a 30-day washout period. Subjects were excluded if they were a current smoker or if they had smoked cigarettes, cigars, a pipe, etc. for 3 months prior to screening and they had to be willing to remain non-smoking for the duration of the study (the use of "vape" pens was allowed). They were further excluded if they had ever had facial cosmetic surgery affecting skin elasticity/flexibility (e.g. face lift, etc.) or if they had Botox® or another facial injection (dermal filler) at or near the temple area within 6 months of screening. Moreover, subjects were excluded if they had undergone any medical procedure that would materially affect the appearance, texture, or elasticity of skin at or near the evaluation site (e.g. chemical peel, laser resurfacing, dermaplaning, microdermabrasion, etc.) within 6 months prior to screening or if they had experienced within 30 days prior to screening any clinically significant confounding disease or condition that would interfere with the study evaluation (e.g. an allergic response resulting in hives or other skin manifestation, an outbreak of rosacea, a severe sunburn, eczema, psoriasis, skin cancer, etc.). Other exclusionary criteria were: body weight 350 pounds (159 kg) or greater, a known allergy to eggs or egg products, or pregnant or breastfeeding women. Subjects previously enrolled in a research study involving an investigational product (drug, device, or biologic) or a new application of an approved product, within 30 days of screening were also excluded from participating in the trial.

### 2.3. Clinical Endpoints/Treatment Response

The primary endpoint for the study was any statistically significant improvement in skin elasticity in the anterior region of the temporal fossa of the face versus placebo evaluated at 12 weeks of treatment based upon measurements taken with the Cutometer® MPA 580 multi-probe system. Secondary endpoints were any statistically significant improvement in skin firmness, viscoelasticity, TEWL or hydration in the anterior region of the temporal fossa of the face versus placebo evaluated at 12 weeks of treatment based upon measurements taken with the Cutometer® MPA 580 multi-probe system. An additional secondary endpoint was any statistically significant improvement in skin appearance, texture or overall health versus placebo evaluated at 12 weeks of treatment determined via a Patient's Assessment. A final secondary endpoint was any statistically significant improvement in hair thickness, texture, overall health, or growth rate or fingernail appearance, strength or growth rate versus placebo evaluated at 12 weeks of treatment as determined via a Patient's Assessment.

### 2.4. Investigational Product

For this clinical study, 400 mg of Ovolux™ powder (Lot#4013260) containing 300 mg of ESM or placebo (300 mg of psyllium husk fiber powder) were provided in #0 vegetarian capsules by ESM Technologies, LLC (Carthage, MO USA). Psyllium husk fiber was chosen as a placebo due to its resemblance to ESM powder and its

lack of effect on skin health. Treatment and placebo capsules were identical in appearance, odor, and taste and were stored in closed containers at ambient temperature. Participants were initially provided a 6-week supply of capsules and were instructed to take one capsule daily with water, approximately the same time each morning with breakfast. An additional 6-week supply of capsules was provided at the Week 6 clinic visit.

### 2.5. Safety/Adverse Events

Secondary objectives of the study were to evaluate the tolerability and safety or any adverse reactions associated with ingestion of ESM powder. Subjects provided a complete medical history and vital signs (resting heart rate, blood oxygenation) were obtained. Additionally, participants' self-assessment diaries were reviewed at each clinic visit and any adverse events were recorded and reported in accordance with applicable FDA regulations. Adverse events and serious adverse events were assessed by the clinical investigator and were followed until resolution, as necessary. Serious adverse events were required to be reported to the clinical monitor immediately.

### 2.6. Statistical Analysis

The hypothesis for this study was that the treatment group would be superior to that of the placebo group in improving skin elasticity over 12 weeks. A 15% absolute change in the mean treatment response (ESM would improve elasticity by an average of at least 15% more in the treatment group than in the placebo group) was expected based upon results from a previously published ESM skin evaluation trial [20]. We therefore estimated that a sample size of 24 subjects would need to be enrolled to provide the study with a statistical power of 80% to detect a clinically meaningful difference between the treatment group and the placebo group, assuming a rate of response of +15% for the treatment group and a rate of response of 0% for the placebo group, with no dropouts. Since the actual enrollment for the study was 63 subjects with one dropout, this should be sufficient to provide adequate safety and comparative effectiveness information. Descriptive statistics were calculated including mean age, height, weight, body mass index (BMI), and blood oxygenation, and comparisons of this demographic data were made with a Kolmogorov-Smirnov test for independent distributions at baseline to validate randomization. Following evaluation for normality (Shapiro-Wilk), post-baseline between-group statistical analyses were done utilizing either an independent group Mann-Whitney test for multiple comparisons for non-parametric data (elasticity, firmness, viscoelasticity, TEWL, hair, skin, and fingernail questionnaires) or an independent group t test for parametric data (hydration). Post-baseline within-group statistical analyses were done utilizing repeated measures analysis of variance (rm-ANOVA). In all cases, statistical significance was accepted at $p < 0.05$. Analysis of the primary endpoint, as well as all secondary endpoints was conducted on the intent-to-treat (ITT) population (*i.e.* including all

randomized patients with at least one efficacy assessment after randomization). The last observation carried forward (LOCF) approach was used for patients who made at least one follow-up visit but who did not complete the study (lost to follow-up). GraphPad Prism® Software (version 10.3.0) was used for all statistical analyses [21].

## 3. Results

Recruitment began in November 2023 at a single clinical site in Missouri and the final follow-up was conducted in March 2024. Sixty-three (63) male and female subjects were screened and a total of 63 subjects between the ages of 35 and 66 years were enrolled in the trial and underwent randomization. Thirty-one subjects (49.2%) were randomized to the placebo group and 32 subjects (50.8%) were randomized to the ESM treatment group (**Figure 1**). All subjects that completed the study did so per the protocol and there was one dropout. Compliance with the study treatment regimen was good in both treatment groups, as judged by capsule count at the final clinic visit. The mean room conditions across baseline, Week 6 and Week 12 were: temperature 23.7˚C, 34.1% RH.

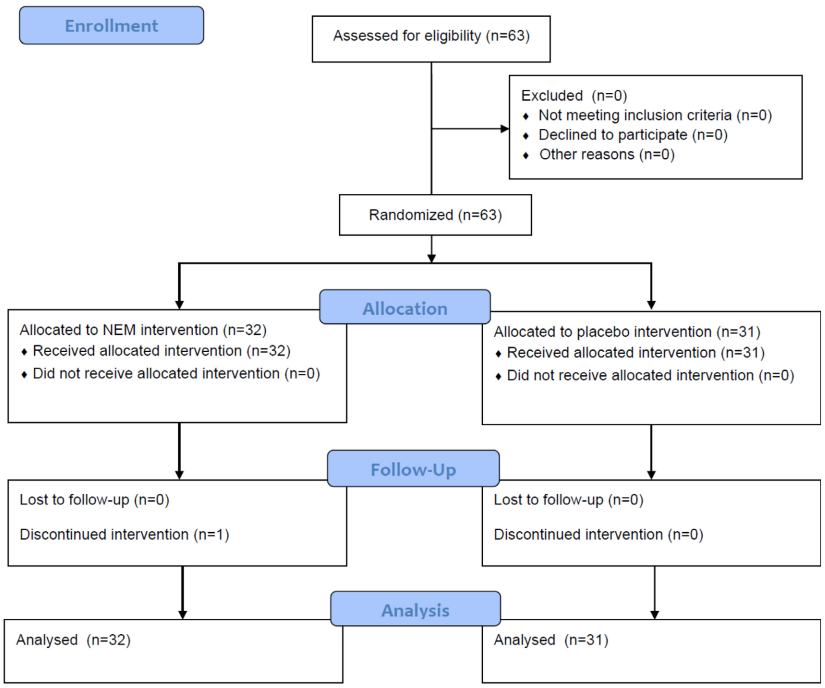



**Figure 1.** A CONSORT trial participant flow diagram for the present study.

### 3.1. Participant Characteristics and Demographics

Participant demographic data (**Table 1**) was initially evaluated to verify randomization. There were no abnormalities in any of these baseline evaluations (not shown). One subject (1.6%) withdrew from the study before the 6-week timepoint; however, their last observation was carried forward and therefore was included in all subsequent data analyses.

**Table 1.** Participant baseline demographic data.

| | Ovolux ESM (n = 32) | Placebo (n = 31) |
|---|---|---|
| Age, yrs | 50.6 ± 9.5 | 48.5 ± 7.4 |
| Sex | | |
| Female (%) | 24 (75) | 19 (61) |
| Male (%) | 8 (25) | 12 (39) |
| Height, cm | 169 ± 10 | 172 ± 11 |
| Weight, kg | 83.4 ± 20.9 | 90.0 ± 18.1 |
| BMI, kg/m$^2$ | 29.0 ± 5.7 | 30.3 ± 6.2 |
| Blood Oxygenation, % | 97.9 ± 1.4 | 97.3 ± 1.2 |

**Notes:** Except where indicated otherwise, values are reported as mean ± standard deviation. Vital signs were obtained after at least 15 minutes, while seated. **Abbreviations:** BMI, body mass index, calculated as weight in kilograms divided by the square of height in meters.

A clinical comparison of valid subjects was carried out to obtain mean scores for both the subjective outcome measures (skin, hair & fingernail questionnaires) and the objective outcome measures (hydration, TEWL & firmness/elasticity) after six weeks and twelve weeks of treatment. Absolute Treatment Effects ($TE_{abs}$) for all outcome measures were calculated as the net difference of treatment versus placebo for the mean change in treatment effect from baseline for each group expressed as a percentage. Positive values represent superior results in the ESM treatment group for hydration, total elasticity, and net elasticity, whereas negative values represent superior results in the ESM treatment group for TEWL, firmness, and viscoelasticity.

### 3.2. Outcome Measures/Endpoints

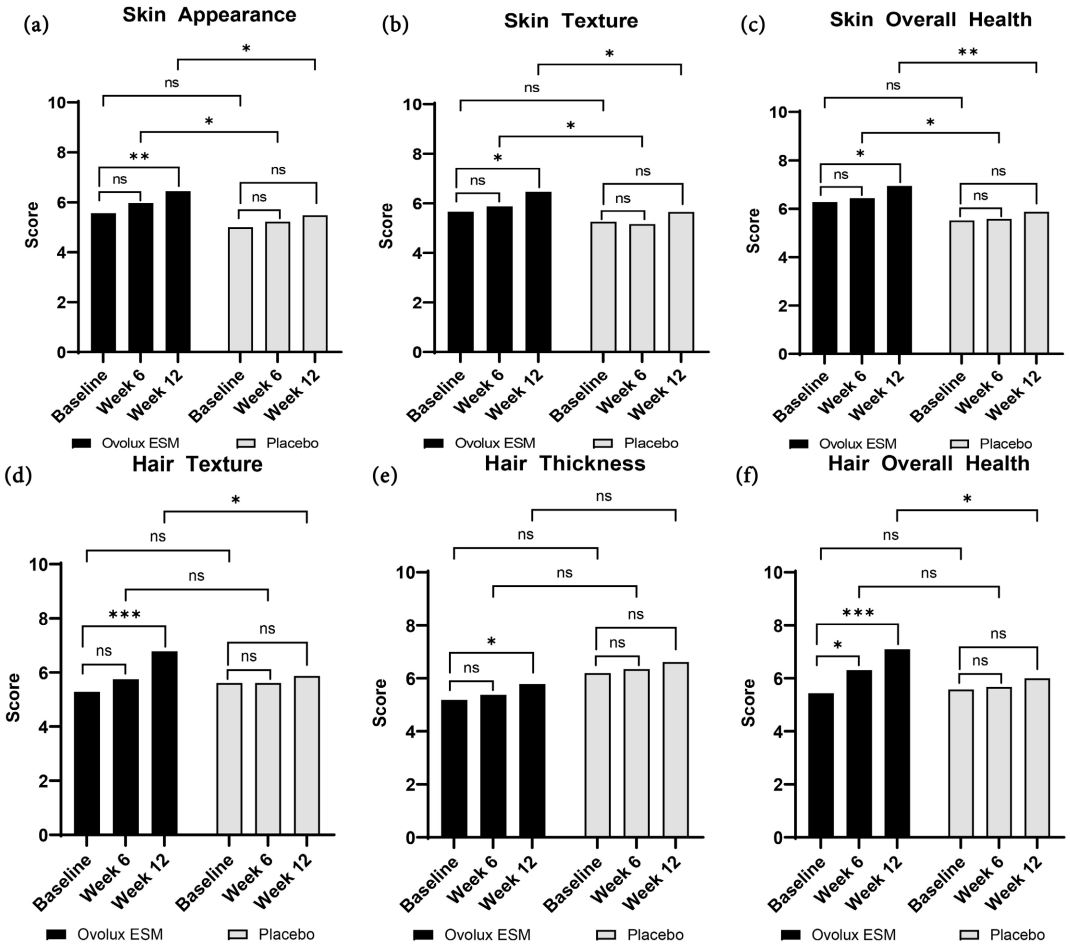
Statistical analysis of the primary outcome measure (total elasticity, R2) revealed that supplementation with ESM produced a significant treatment response versus placebo after both six weeks ($TE_{abs}$ +8.4%, $p = 0.019$) and twelve weeks of treatment ($TE_{abs}$ +12.2%, $p = 0.002$) (Table 2). Within-group improvement from baseline was also statistically significant for total elasticity for the ESM treatment group at both timepoints (Week 6, +8.0%, $p = 0.001$; Week 12, +11.2%, $p < 0.001$), whereas neither timepoint was significant for the placebo group. There were also significant treatment responses versus placebo for firmness (R0), net elasticity (R5), and viscoelasticity (R6) at both timepoints (R0: Week 6, $TE_{abs}$ −23.9%, $p < 0.001$; Week 12, $TE_{abs}$ −16.8%, $p = 0.002$) (R5: Week 6, $TE_{abs}$ +9.5%, $p = 0.013$; Week 12, $TE_{abs}$ +10.6%, $p = 0.032$) (R6: Week 6, $TE_{abs}$ -17.9%, $p < 0.001$; Week 12, $TE_{abs}$ −17.7%, $p < 0.001$). Within-group improvement from baseline was also statistically significant for firmness (R0) at Week 6 (+7.4%, $p = 0.006$), whereas the placebo group worsened significantly from baseline at both timepoints (Week 6, −16.5%, $p < 0.001$; Week 12, −13.2%, $p < 0.001$). Within-group improvement from

baseline was also significant for net elasticity (R5) for the ESM treatment group at both timepoints (Week 6 +10.8%, p = 0.002; Week 12 +10.6%, p = 0.008), whereas neither timepoint was significant for the placebo group. Viscoelasticity (R6) also improved significantly within-group at both timepoints for the ESM treatment group (Week 6, −10.7%, p = 0.002; Week 12, −10.0%, p = 0.005), while the placebo group significantly worsened at both timepoints (Week 6, +7.2%, p = 0.031; Week 12, +7.7%, p = 0.025). There was a moderate significant absolute treatment effect for skin hydration versus placebo at both 6 weeks ($TE_{abs}$ +8.3%, p = 0.010) and 12 weeks ($TE_{abs}$ +8.6%, p = 0.019) of treatment. The skin hydration with ESM treatment decreased modestly within-group by Week 6 (−5.6%, p = 0.040) but essentially recovered by Week 12 (−0.9%, p = 0.850), whereas the placebo group significantly worsened from baseline at both timepoints (Week 6, −15.4%, p < 0.001; Week 12, −10.5%, p = 0.010). TEWL was not significantly different from placebo at either timepoint and there were no significant within-group changes for either treatment group.

**Table 2.** Mean skin hydration, TEWL, firmness, total elasticity, net elasticity, and viscoelasticity as measured with a Cutometer® MPA 580 in Ovolux ESM-treated and placebo groups at baseline and after 6 weeks and 12 weeks of supplementation.

| | Time Post-Dose | Treatment Ovolux ESM | Absolute Placebo | Treatment Effect | P-value (Tx vs Pbo) |
|---|---|---|---|---|---|
| Hydration | Baseline (n = 32, 31) | 43.4 ± 10.6 | 41.7 ± 12.0 | - | 0.548 |
| | 6 Weeks (n = 32, 31) | 41.0 ± 12.2* | 35.3 ± 11.0** | +8.3% | 0.010 |
| | 12 Weeks (n = 32, 31) | 43.0 ± 10.1 | 37.3 ± 10.1* | +8.6% | 0.019 |
| TEWL, g/m²/h | Baseline (n = 32, 31) | 19.1 ± 6.8 | 17.3 ± 4.0 | - | 0.519 |
| | 6 Weeks (n = 32, 31) | 18.1 ± 7.3 | 16.6 ± 5.0 | −1.2% | 0.730 |
| | 12 Weeks (n = 32, 31) | 18.6 ± 5.5 | 17.3 ± 5.1 | −2.6% | 0.289 |
| Firmness, mm (R0) | Baseline (n = 32, 31) | 0.363 ± 0.084 | 0.330 ± 0.060 | - | 0.375 |
| | 6 Weeks (n = 32, 31) | 0.336 ± 0.066** | 0.385 ± 0.076** | −23.9% | < 0.001 |
| | 12 Weeks (n = 32, 31) | 0.350 ± 0.073 | 0.374 ± 0.077** | −16.8% | 0.002 |
| Total Elasticity, % (R2) | Baseline (n = 32, 31) | 75.1 ± 12.0 | 77.6 ± 10.7 | - | 0.377 |
| | 6 Weeks (n = 32, 31) | 81.1 ± 11.4** | 77.3 ± 9.6 | +8.4% | 0.019 |
| | 12 Weeks (n = 32, 31) | 83.5 ± 8.8** | 76.8 ± 9.1 | +12.2% | 0.002 |
| Net Elasticity, % (R5) | Baseline (n = 32, 31) | 82.2 ± 24.4 | 86.1 ± 22.3 | - | 0.414 |
| | 6 Weeks (n = 32, 31) | 90.0 ± 22.3* | 87.2 ± 15.6 | +9.5% | 0.013 |
| | 12 Weeks (n = 32, 31) | 89.8 ± 24.3** | 86.1 ± 19.1 | +10.6% | 0.032 |
| Viscoelasticity, % (R6) | Baseline (n = 32, 31) | 49.2 ± 8.3 | 47.1 ± 9.4 | - | 0.345 |
| | 6 Weeks (n = 32, 31) | 43.9 ± 9.3** | 50.5 ± 9.3* | −17.9% | < 0.001 |
| | 12 Weeks (n = 32, 31) | 44.3 ± 9.4** | 50.7 ± 9.8* | −17.7% | < 0.001 |

Except where indicated otherwise, values are reported as mean ± standard deviation (SD). **P < 0.01, *P < 0.05 within-group versus baseline. TEWL = trans-epidermal water loss. Tx = treatment, Pbo = placebo




***P < 0.001, **P < 0.01, *P < 0.05, ns = not significant.

**Figure 2.** Mean skin appearance (a), texture (b), and overall health (c), and hair thickness (d), texture (e), and overall health (f) questionnaire scores in Ovolux ESM-treated and placebo groups at baseline and after 6 weeks and 12 weeks of supplementation.

There were statistically significant improvements in the ESM treatment group versus placebo for skin appearance, texture, and overall health as determined by patient assessments (**Figure 2(a-c)**). There was a modest but significant absolute treatment effect for skin appearance versus placebo at both 6 weeks ($TE_{abs}$ +2.8%, p = 0.045) and 12 weeks ($TE_{abs}$ +6.1%, p = 0.028) of treatment. Within-group improvement from baseline was also significant for skin appearance for the ESM treatment group for Week 12 (+15.7%, p = 0.002), whereas the placebo group results were non-significant at both timepoints. Similarly, there was a moderate significant absolute treatment effect for skin texture versus placebo at both 6 weeks ($TE_{abs}$ +5.7%, p = 0.049) and 12 weeks ($TE_{abs}$ +7.0%, p = 0.033) of treatment. Within-group improvement from baseline was also significant for skin texture for the ESM treatment group for Week 12 (+14.4%, p = 0.028), whereas the placebo group results were non-significant at both timepoints. There was additionally a small but significant absolute treatment effect for overall skin health versus placebo at both 6 weeks ($TE_{abs}$ +1.3%, p = 0.010) and 12 weeks ($TE_{abs}$

+4.0%, p = 0.010) of treatment. Within-group improvement from baseline was also significant for overall skin health for the ESM treatment group for Week 12 (+10.4%, p = 0.032), whereas the placebo group results were non-significant at both timepoints.

There were also statistically significant improvements in the ESM treatment group versus placebo for hair texture and overall hair health as determined by patient assessments (**Figure 2(e-f)**). Hair thickness was not significantly different from placebo at either timepoint, despite the fact that there was a statistically significant within-group improvement from baseline for the ESM treatment group for Week 12 (+11.4%, p = 0.036), whereas the placebo group results were non-significant at both timepoints. There was a significant absolute treatment effect for hair texture versus placebo at 12 weeks ($TE_{abs}$ +23.8%, p = 0.026) of treatment. Within-group improvement from baseline was also significant for hair texture for the ESM treatment group for Week 12 (+28.4%, p < 0.001), whereas the placebo group results were non-significant at both timepoints. There was additionally a significant absolute treatment effect for overall hair health versus placebo at 12 weeks ($TE_{abs}$ +22.9%, p = 0.027) of treatment. Within-group improvement from baseline was also significant for overall hair health for the ESM treatment group at both 6 weeks (+16.1%, p = 0.033) and 12 weeks (+30.5%, p < 0.001) of treatment, whereas the placebo group results were non-significant at both timepoints. Hair growth rate was not significantly different from placebo at either timepoint, despite the fact that there was a statistically significant within-group improvement from baseline for the ESM treatment group for Week 12 (+19.8%, p = 0.013), whereas the placebo group results were non-significant at both timepoints.

Fingernail appearance was not significantly different from placebo at either timepoint. There was, however, a statistically significant within-group improvement from baseline for the ESM treatment group for Week 12 (+25.7%, p = 0.005). Similarly, fingernail strength was not significantly different from placebo at either timepoint. Nonetheless, there was a statistically significant within-group improvement from baseline for the ESM treatment group for Week 12 (+15.7%, p = 0.040). Fingernail growth rate was also not significantly different from placebo at either timepoint and there were no within-group changes in either treatment group. Alternatively, evaluating the fingernail data for females only, resulted in significant absolute treatment effects for appearance ($TE_{abs}$ +6.7%, p = 0.059), strength ($TE_{abs}$ +10.0%, p = 0.035), and growth rate ($TE_{abs}$ +3.8%, p = 0.020) versus placebo at 12 weeks.

### 3.3. Safety Outcome

Throughout the twelve-week study, there were forty-three (43) adverse events (AEs) reported by the ESM treatment group, the most common of which were eighteen (18) instances of cold/flu/sinus congestion and ten (10) instances of headache. None of the treatment group AEs were judged by the clinical investigator to be associated with treatment. There were forty (40) AEs reported by the

placebo group, the most common of which were thirteen (13) instances of headache and twelve (12) instances of cold/flu/sinus congestion. None of the placebo group AEs were judged by the clinical investigator to be associated with treatment. There was no obvious difference in the pattern of AEs between the two treatment groups and there were no serious AEs reported in either treatment group. The treatment was reported to be well tolerated by study participants.

## 4. Discussion

Because ESM closely resembles the ECM constituents of human skin, it is not particularly surprising that ESM has shown significant benefits when used topically for wound healing [22] [23]. Perhaps more interesting is that oral supplementation with ESM has proven so impactful on the health of skin, the so-called "beauty from within" effect. The present study not only confirmed the total elasticity results from prior pilot studies [18] [19], it extended these beneficial effects to the objective measures of skin hydration, firmness, net elasticity, and viscoelasticity, as well as the subjective measures of skin appearance, skin and hair texture, and skin and hair health overall. And in females only, there were additional benefits for subjective measures of fingernail strength and growth rate.

Ovolux™ ESM provided significant improvement (+8.4%) versus placebo in the primary outcome measure total elasticity (R2) after just 6 weeks, and this benefit increased to +12.2% by the end of the study (12 weeks). ESM also produced similar improvements in net elasticity (R5) (+9.5% and +10.6%, respectively), viscoelasticity (R6) (−17.9% and −17.7%, respectively), and firmness (R0) (−23.9% and −16.8% respectively). Collectively, these broad-based effects on critical biomechanical skin properties demonstrate the naturally occurring, multi-faceted approach to ameliorating the clinical signs of aging that results from oral supplementation with ESM. Accordingly, ESM helps skin to resist epidermal thickening (*i.e.* it maintains elasticity), to resist dermal thinning (*i.e.* it maintains viscoelasticity), and to resist skin laxity and elastosis (*i.e.* it maintains firmness) found in intrinsically aging skin. These effects likely arise from calcium signaling and GP6 signaling pathways that were shown to be substantially upregulated by oral supplementation with ESM in a mouse model of skin aging [16]. The latter pathway is particularly important as it plays a crucial role in collagen synthesis, and Furukawa, *et al.* found a 2-fold to 3-fold upregulation of the collagen synthesis genes Col1a1 and Col2a1, the fibroblast growth factor gene Fgf1, and the hyaluronic acid synthesis gene Has2, along with numerous others [16].

ESM also supplied a benefit for skin hydration during the study (+8.3% and +8.6%, respectively). This demonstrated meaningful protection against the drying effects from winter weather (the season during which the present study was conducted) as the placebo group skin hydration dropped by over 10% during the course of the study, whereas the ESM treatment group skin hydration was essentially unaffected. ESM did not provide a benefit for the related parameter TEWL in the present study. Interestingly, this is consistent with what Gil-Quintana, *et al.*

found for TEWL in their dermal study of ESM [20]. It isn't clear why ESM would maintain hydration without a concomitant effect on TEWL, particularly in light of the effects observed by Sim, *et al.* in photoaged skin of mice [17]. It could be that the treatment effect was just too small to be significant in this and previous studies versus the >100% deficit in TEWL produced in the Sim, *et al.* study design. It could also be related to the fact that the room conditions in the present study (23.7˚C, 34.1% RH) were warmer and drier than the conditions recommended by the Tewameter® TM300 probe manufacturer (20 - 22˚C, 40% - 60% RH).

The objective results determined with the various Cutometer® MPA 580 probes are of obvious importance, however it is also critical to demonstrate that these measures translate to the subjective perceptions of the participants. This was indeed the case in the present study, as subjects found noticeable improvements in the appearance, texture, and overall health of their skin compared to placebo subjects at both 6 weeks (+2.8%, +5.7%, +1.3%, respectively) and 12 weeks (+6.1%, +7.0%, +4.0%, respectively). This confirms that the improvements in skin biomechanical properties from ESM supplementation determined with the Cutometer® are large enough to be perceived by the study participants.

Similar benefits were observed for hair texture and overall health at 12 weeks (+23.8% and +22.9%, respectively). It is consistent that skin would benefit earlier from oral ESM than would an associated appendage like hair, that is derived from differentiated keratinocytes. Human hair grows roughly 1.1 cm per month [24], which could make it difficult for participants to evaluate not only growth rate but also thickness and texture in just 6 weeks. This would reasonably explain the lack of significance at 6 weeks versus placebo, despite a significant (+16.1%) within-group improvement in overall hair health in the ESM treatment group at 6 weeks.

Although fingernail improvements were not noted in the treatment group overall versus placebo, there were some benefits in females only for strength and growth rate at 12 weeks (+10.0% and +3.8%, respectively). Again, it is consistent that skin would benefit earlier from oral ESM than would an associated appendage like fingernails. Human fingernails grow roughly 3 mm per month [25], which could make it difficult for participants to evaluate appearance, strength, and growth rate in just 3 months. It is unclear why this effect would only be observed in female subjects, however this may be due to the fact that these subjects are often more attentive to the condition of their fingernails than are their male counterparts.

There was a moderate number of adverse events (AEs) in the trial, all of which were deemed unrelated to treatment, and no serious AEs occurred. Although there were a greater number of AEs in the ESM group (52%) versus the placebo group (48%), there were no obvious trends in these occurrences. The vast majority of AEs in both groups were related to cold/flu/sinus congestion and headaches, which is consistent with a study conducted during the height of cold and flu season in the U. S. No side effects were noted in this trial. Food-derived natural products such as eggshell membrane would be expected to have a robust safety profile,

and this appears to be borne out in this study.

One limitation of the present study was that subjects had healthy hair, skin, and fingernails. That is, there were a moderate number of subjects in both the ESM and placebo (pbo) groups for whom their hair, skin, and fingernails were sufficiently healthy that they couldn't improve (hair, 11 subjects [6 ESM, 5 pbo]; skin, 13 subjects [8 ESM, 5 pbo]; fingernails, 9 subjects [3 ESM, 6 pbo]). These subjects were, in essence, non-responders. In practical terms, this reduced the study size by 14% - 21%, which would in turn reduce the sensitivity of the questionnaires to detect differences. This could partially explain why hair and fingernail changes weren't detected until the end of the study, at 12 weeks. Also of note, nearly all of these subjects were age 50 years or younger. Future investigations should probably limit the subject population to 45 years and older.

## 5. Conclusion

Ovolux™ ESM, 400 mg daily, provided significant broad-based improvements in critical biomechanical skin properties versus placebo, including elasticity, firmness, and viscoelasticity. These effects, along with improvement in skin hydration, were observed as early as 6 weeks and continued through the end of the 12-week study. Moreover, there were significant improvements versus placebo in skin appearance, skin and hair texture, and skin and hair health overall, as well as fingernail strength and growth rate in females only. Treatment with ESM was reported to be well-tolerated. Ovolux™ ESM appears to be a natural solution to help skin to resist the epidermal thickening, the dermal thinning, and the skin laxity and elastosis found in intrinsically aging skin.

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## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

## References

[1] Department of Economic and Social Affairs (2023) World Social Report 2023: Leaving No One Behind in an Ageing World.

[2] Wong, Q.Y.A. and Chew, F.T. (2021) Defining Skin Aging and Its Risk Factors: A Systematic Review and Meta-Analysis. *Scientific Reports*, **11**, Article No. 22075. https://doi.org/10.1038/s41598-021-01573-z

[3] Sampaio, A.L., Bressan, A.L., Vasconcelos, B.N. and Gripp, A.C. (2021) Skin Manifestations Associated with Systemic Diseases—Part I. *Anais Brasileiros de Dermatologia*, **96**, 655-671. https://doi.org/10.1016/j.abd.2021.02.008

[4] Leal, J.M., de Souza, G.H., Marsillac, P.F.D. and Gripp, A.C. (2021) Skin Mani-

festations Associated with Systemic Diseases—Part II. *Anais Brasileiros de Dermatologia*, **96**, 672-687. https://doi.org/10.1016/j.abd.2021.06.003

[5] Kolarsick, P.A.J., Kolarsick, M.A. and Goodwin, C. (2011) Anatomy and Physiology of the Skin. *Journal of the Dermatology Nurses' Association*, **3**, 203-213. https://doi.org/10.1097/jdn.0b013e3182274a98

[6] Oh, J., Kim, Y.K., Jung, J., Shin, J. and Chung, J.H. (2011) Changes in Glycosaminoglycans and Related Proteoglycans in Intrinsically Aged Human Skin *in Vivo*. *Experimental Dermatology*, **20**, 454-456. https://doi.org/10.1111/j.1600-0625.2011.01258.x

[7] Baumann, L. (2007) Skin Ageing and Its Treatment. *The Journal of Pathology*, **211**, 241-251. https://doi.org/10.1002/path.2098

[8] Papanagiotou, V.D. (2009) Skin Aging and Photoaging. *Dermattikon*, **4**, 57-65.

[9] Helfrich, Y.R., Sachs, D.L. and Voorhees, J.J. (2008) Overview of Skin Aging and Photoaging. *Dermatological Nursing*, **20**, 177-183.

[10] Wong, M., Hendrix, M.J.C., von der Mark, K., Little, C. and Stern, R. (1984) Collagen in the Egg Shell Membranes of the Hen. *Developmental Biology*, **104**, 28-36. https://doi.org/10.1016/0012-1606(84)90033-2

[11] Arias, J.L., Fernandez, M.S., Dennis, J.E. and Caplan, A.I. (1991) Collagens of the Chicken Eggshell Membranes. *Connective Tissue Research*, **26**, 37-45. https://doi.org/10.3109/03008209109152162

[12] Makkar, S., Liyanage, R., Kannan, L., Packialakshmi, B., Lay, J.O. and Rath, N.C. (2015) Chicken Egg Shell Membrane Associated Proteins and Peptides. *Journal of Agricultural and Food Chemistry*, **63**, 9888-9898. https://doi.org/10.1021/acs.jafc.5b04266

[13] Ahmed, T.A.E., Suso, H. and Hincke, M.T. (2017) In-Depth Comparative Analysis of the Chicken Eggshell Membrane Proteome. *Journal of Proteomics*, **155**, 49-62. https://doi.org/10.1016/j.jprot.2017.01.002

[14] Liu, Z., Sun, X., Cai, C., He, W., Zhang, F. and Linhardt, R.J. (2016) Characteristics of Glycosaminoglycans in Chicken Eggshells and the Influence of Disaccharide Composition on Eggshell Properties. *Poultry Science*, **95**, 2879-2888. https://doi.org/10.3382/ps/pew179

[15] Arias, J., Carrino, D.A., Fernández, M.S., Rodríguez, J.P., Dennis, J.E. and Caplan, A.I. (1992) Partial Biochemical and Immunochemical Characterization of Avian Eggshell Extracellular Matrices. *Archives of Biochemistry and Biophysics*, **298**, 293-302. https://doi.org/10.1016/0003-9861(92)90126-h

[16] Furukawa, K., Kono, M., Kataoka, T., Hasebe, Y., Jia, H. and Kato, H. (2021) Effects of Eggshell Membrane on Keratinocyte Differentiation and Skin Aging *in Vitro* and *in Vivo*. *Nutrients*, **13**, 2144. https://doi.org/10.3390/nu13072144

[17] Sim, W., Ahn, J., Lim, W., Son, D.J., Lee, E. and Lim, T. (2022) Anti-skin Aging Activity of Eggshell Membrane Administration and Its Underlying Mechanism. *Molecular & Cellular Toxicology*, **19**, 165-176. https://doi.org/10.1007/s13273-022-00291-5

[18] Aguirre, A., Gil-Quintana, E., Fenaux, M., Erdozain, S. and Sarria, I. (2017) Beneficial Effects of Oral Supplementation with Ovoderm on Human Skin Physiology: Two Pilot Studies. *Journal of Dietary Supplements*, **14**, 706-714. https://doi.org/10.1080/19390211.2017.1310781

[19] Ohto-Fujita, E., Hatakeyama, N., Atomi, A., Yasuda, S., Kodama, S., Atomi, T., *et al*. (2021) Effect of Eggshell Membrane Powder Intake on the Body Function of Healthy Individuals. *Journal of Fiber Science and Technology*, **77**, 258-265. https://doi.org/10.2115/fiberst.2021-0029

[20] Aguirre, A., Gil-Quintana, E. and La Nuez, M. (2018) Supplementation with Ovoderm® Reduces the Clinical Signs of Skin Aging. a Double-Blind, Placebo-Controlled Study. *Journal of Clinical Research in Dermatology*, **5**, 1-8. https://doi.org/10.15226/2378-1726/5/2/00180

[21] GraphPad Prism. https://www.graphpad.com

[22] Yang, J.Y., Chuang, S.S., Yang, W.G. and Tsay, P.K. (2003) Egg Membrane as a New Biological Dressing in Split-Thickness Skin Graft Donor Sites: A Preliminary Clinical Evaluation. *Chang Gung Medical Journal*, **26**, 153-159.

[23] Ahmed, T.A.E., Suso, H., Maqbool, A. and Hincke, M.T. (2019) Processed Eggshell Membrane Powder: Bioinspiration for an Innovative Wound Healing Product. *Materials Science and Engineering: C*, **95**, 192-203. https://doi.org/10.1016/j.msec.2018.10.054

[24] Loussouarn, G., Lozano, I., Panhard, S., Collaudin, C., El Rawadi, C. and Genain, G. (2016) Diversity in Human Hair Growth, Diameter, Colour and Shape. An *in Vivo* Study on Young Adults from 24 Different Ethnic Groups Observed in the Five Continents. *European Journal of Dermatology*, **26**, 144-154. https://doi.org/10.1684/ejd.2015.2726

[25] De Berker, D.A.R., André, J. and Baran, R. (2007) Nail Biology and Nail Science. *International Journal of Cosmetic Science*, **29**, 241-275. https://doi.org/10.1111/j.1467-2494.2007.00372.x