

A botanically derived skin surface lipid mimetic based on the composition of healthy 22-year-old females

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Synopsis

Introduction: Skin surface lipids (SSLs) greatly affect the skin physiology and are thought to be involved in skin processes such as thermoregulation, bacterial colonization, and barrier function and maintenance. SSLs are primarily composed of fatty acids, triglycerides, cholesterol, steryl esters, wax esters, and squalene. The objective of this research was to evaluate and better understand the SSL composition and variation in an age- and sex-controlled population, and create an appropriate botanically derived mimetic. **Methods:** SSL samples taken from the foreheads of 59 healthy, 22-year-old females were analyzed by gas chromatography mass spectrometry (GC-MS). Using botanically derived raw materials from *Macadamia integrifolia*, *Simmondsia chinensis*, and *Olea europaea*, a mimetic was engineered via a series of esterification reactions and lipid components quantitated with GC-MS. The glyceride and wax ester components were produced by the interesterification of *M. integrifolia* and *S. chinensis* under specified conditions. The steryl ester component was produced by the esterification of the fatty acids of *M. integrifolia* and phytosterols under similar conditions. **Results:** The following major classes of lipids were found and quantified by percent composition: glycerides, free fatty acids, squalene, wax esters, steryl esters, and cholesterol. The variability between subjects for each component was minimal; however, the greatest variation was seen for free fatty acids and cholesterol. Correlations among the components were calculated and found to be statistically or directionally significant with few exceptions. The esterification reactions of jojoba, macadamia, and tall oils, along with a precise addition of squalene derived from *O. europaea*, produced a suitable SSL mimetic. When applied to delipidized skin, the mimetic helped restore barrier function, increased skin hydration, and increased skin elasticity and firmness in aged skin. **Discussion:** The present research indicates that, overall, the SSL composition is quite consistent in a controlled population of 22-year-old females. Furthermore, there were strong correlations between the SSL components among subjects, with the exception of squalene and steryl esters. This was expected due to the fact that of the six major SSL components, steryl esters and squalene also showed higher variation over time for each individual. The variation in free fatty acids may be attributable to the potential differences in the microflora of the subjects. The variation in this study's results, as compared to previously published work, could indicate that the collection methods, geographic location, gender, and age specificity contribute to the distribution or collection of different lipid components on the skin surface. Since the excretion of sebum is known to decrease in females after 40 years of age, the proposed mimetic could be a beneficial supplement to human SSLs in aged skin, as well as in skin where the stratum

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corneum is defective, by aiding in the restoration of barrier function, while increasing skin hydration, elasticity, and firmness.

INTRODUCTION

Skin surface lipids (SSLs) are primarily composed of fatty acids (FAs), triglycerides, cholesterol, cholesteryl esters, wax esters, and squalene (1). The SSLs are either of sebaceous origin (i.e., sebum) (approximately 90%) or epidermal origin (approximately 10%), and the fraction of any one component varies based on body location (i.e., whether the location is rich in sebaceous glands) (2). Wax esters, squalene, glycerides, free fatty acids (FFAs), and cholesteryl esters are primarily derived from sebaceous glands (3). Cholesterol, FFA, and ceramides mainly originate in the epidermis along with small amounts of cholesteryl esters, and glycerides (3). SSLs greatly affect the skin physiology and are thought to be involved in skin processes such as thermoregulation (4), bacterial colonization (5), and barrier function and maintenance (6).

Although much research has been done to quantify the total content and various SSL components, there is still much debate as to the best method of noninvasive collection and analytical technique, as well as whether or not outside factors such as race, gender, and age affect the collective quantity or the percent composition of each SSL component. Previously explored methods of collection include (i) solvent extraction (2), (ii) Sebutape[®] (CuDerm Corporation, Dallas, TX) (7), and (iii) cigarette paper (8). Additionally, samples have been analyzed using various methods such as (i) thin layer chromatography (8), (ii) infrared spectroscopy (9), (iii) high-temperature gas chromatography and mass spectrometry (GC-MS) (10), (iv) nuclear magnetic resonance spectroscopy (11), (v) high-performance liquid chromatography (HPLC) and MS (12), or (vi) combinations of analytical techniques (13).

This current body of research used the cigarette paper collection method in conjunction with GC-MS analysis to determine the percent composition of each of the SSL components. On the basis of the research conducted by Shetage *et al.* (14), the cigarette paper method produces the most consistent data. Analysis by GC-MS allows optimal separation and identification of desired lipid components and constituents that cannot be achieved without a combination of chromatographic and mass spectral analysis. An all-female population was used to evaluate variance between highly similar individuals. The age 22 was chosen because sebum excretion rates are at a maximum within the 16- to 40-year age range (15), and remain steady through the 20s and 30s (16).

The composition of 22-year-old healthy female SSLs was then used to generate a botanically sourced SSL mimetic to determine the physiological effects when an SSL mimetic is applied to the skin topically. Moisturizers are often used for treatment of dry skin conditions, whether it is in conjunction with a drug for disease states like atopic dermatitis or psoriasis, daily use for self-perceived dry skin due to age or climate, or as a protectant in the workplace due to frequent contact with chemical agents (17). Moisturizers have both short- and long-term hydration and barrier function effects on the skin, and these effects are highly dependent upon the physicochemical properties of the moisturizer, e.g., pH, occlusivity, and type of ingredients used (17,18). The efficacy portion of this research primarily focused on the short- and long-term functions of skin hydration and skin barrier function and maintenance when an SSL mimetic is applied topically to the skin.

MATERIALS AND METHODS

SUBJECTS AND SAMPLE COLLECTION

The study, which was performed in Chandler, AZ, was approved by the Argus Independent Review Board (Tucson, AZ) prior to beginning any study procedures. Written informed consent was obtained from all subjects. Fifty-nine healthy, 22-year-old females were selected according to the following criteria: no active skin diseases on the face (e.g., acne, psoriasis, atopic dermatitis, eczema, rosacea, and skin cancer), no immunological disorders, and not pregnant or nursing. The subjects consisted of fifty Caucasian, three African American, two Asian, and four mixed race persons.

Solvent-washed cigarette rice papers (Rizla UK, Ltd, Pontypridd, United Kingdom) were used as lipid-free absorbent papers. Among the papers tested, it was found that adhesive-free rice paper contained the least amount of contaminants, particularly lipids. This is often an issue when using adhesive-containing collection methods such as Sebutape (CuDerm Corporation, Dallas, TX). Approximately 50 papers were washed at a time with 250 ml HPLC-grade diethyl ether in an ultrasonic water bath for 15 min at room temperature. After extraction, the cigarette rice papers were removed from the solvent and dried in a rotary evaporator. The papers were stored in polyethylene jars until further use on subjects.

Subjects were instructed to wash their faces approximately 12 h before sampling using Cetaphil[®] Gentle Skin Cleanser (Galderma, Fort Worth, TX), supplied by Floratech (Chandler, AZ), to remove dirt and oil from the facial skin. After this 12-h period, subjects reported to the testing facility where they acclimated in a controlled environment [20°–22°C, <50% relative humidity (RH)]. Noninvasive sampling of SSLs from the forehead of each subject was then conducted over the course of 2 h in the following manner. Two sheets of lipid-free absorbent paper were placed on top of one another in the center of the forehead and held in position for 30 min. The paper was then removed, and placed in a sealed container for extraction. The lipid absorption step was then repeated, consecutively, three additional times.

EXTRACTION AND SAMPLE ANALYSIS

Each subject's collection papers were extracted twice with 25 ml HPLC-grade diethyl ether (Honeywell Burdick and Jackson, Muskegon, MI) in an ultrasonic water bath for 10 min. The collection papers were removed from the extraction flask, and the diethyl ether was evaporated under a gentle stream of nitrogen at 70°C on a hot plate until dry. After drying, the samples were weighed and diluted with an appropriate amount of iso-octane (EMD Millipore, Billerica, MA) to obtain a uniform concentration range for the series of samples, approximately 0.5–0.7 µg/µl.

The instrument consisted of an Agilent 6890 GC with a programmable cool on-column injector coupled to an Agilent 5973N with turbo pump (Santa Clara, CA). The instrumental analysis methods were developed using strategies referenced from Michael-Jubeli *et al.* (19). Software control and data analysis were accomplished with Agilent MSD Chemstation D.02.00.237, NIST 11 (Gaithersburg, MD), and AMDIS v2.70 (Gaithersburg, MD).

SKIN SURFACE LIPID MIMETIC FORMULATION

Refined jojoba oil, macadamia oil, ethyl macadamiate (Floratch), squalene (Ekiz, Izmir, Turkey), and phytosterols (ADM Nutrition, Decatur, IL) were used to formulate the mimetic. The jojoba and macadamia oil underwent a transesterification process under typical conditions in order to distribute the palmitoleic acid among both the wax-ester and triglyceride portions of the material. Ethyl macadamiate and phytosterols were transesterified in a similar process to create a phytosteryl ester of macadamia FAs thereby incorporating palmitoleic acid in the phytosteryl ester portion of the mimetic. The transesterified products of wax esters, triglycerides, and phytosteryl esters along with squalene and phytosterols were mixed in specific quantities resembling SSLs in the final formulation.

EFFICACY ANALYSIS OF TOPICAL APPLICATION OF SSL MIMETIC

Four efficacy studies were conducted in Chandler. Independent review board approval and a written informed consent from each subject were obtained before any protocol-related procedures were undertaken. All study participants were healthy females. Upon arriving at the testing facility, subjects acclimated for 30 min in a controlled environment (20°–22°C, <50% RH). All studies were carried out in a double-blind, vehicle-controlled, randomized manner according to the testing matrix (see Table I). The vehicle contained the following: water (q.s.), methylisothiazolinone and caprylyl glycol (0.9%), ammonium acryloyldimethyltaurate/VP copolymer (0.6%), sorbitan and sucrose cocoate (0.5%), hydroxyethylcellulose (0.3%), and disodium ethylenediaminetetraacetic acid (0.1%). The SSL mimetic was compared to olive oil (OO) and caprylic/capric triglyceride oil (CCT) because these also contain skin-lipid-like components.

RESULTS

SKIN SURFACE LIPID COMPOSITION AND COMPARISON

The initial portion of this study was to evaluate variation in the SSL composition within a population where age and sex were controlled. The sensitivity of GC-MS analysis allows

Table I
Efficacy Testing Matrix

Function	Washout (days)	Anatomical location	<i>n</i>	Sex	Instrument ^a	Insult/condition
Short-term barrier recovery	2	Volar forearms	14	M/F	Tewameter TM 300	Acetone exposure
Short-term hydration	2	Lower legs	12	F	Corneometer [®] CM 825	Dry skin
Long-term skin hydration and barrier function	3	Lower legs	18	F	Corneometer CM 825/Tewameter TM 300	Dry skin
Viscoelasticity and hydration	2	Forearms	13	M/F	MPA Cutometer/Corneometer CM 825	Aged/sun-damaged skin (60–80 years of age)

^aAll instruments are products of Courage + Khazaka (Köln, Germany).

Table II
Mean Percent Composition for SSLs and SSL Mimetic

SSL component	Mean percent composition \pm standard deviation	Mean percent composition of SSL mimetic
Squalene	15.6 \pm 4.8	14.10
Wax esters	15.2 \pm 3.2	18.40
Cholesterol (phytosterol)	0.6 \pm 0.4	0.40
Cholesteryl esters (steryl esters)	2.1 \pm 0.6	1.90
Glycerides	50.3 \pm 12.5	
FFAs	16.2 \pm 10.2	
Glycerides and FFAs	66.5 \pm 6.2	65.20

for differentiation of not only primary SSLs but also differentiation and quantification of constituent FAs. The data in Table II demonstrate the mean percent composition of each SSL component, as well as the skin surface composition of the SSL mimetic. The most abundant component of SSLs was the glycerides. Relatively speaking, of the six SSL components evaluated, FFAs and cholesterol varied the most between subjects; however, when glycerides and FFAs were combined, the variation of the combination decreased greatly compared to the variation of either individual component.

For the purpose of correlation analysis, glycerides and FFA were combined due to the variation in the degree of hydrolysis of triglycerides by bacteria (20). Triglycerides are broken down into diglycerides, monoglycerides, and FFA; therefore, the variability between these components is highly dependent on the microflora of the individual (16). Correlations between the five components were calculated yielding statistically ($p < 0.05$) or directionally ($p < 0.10$) significant correlations between all components with the exception of the following: squalene and wax esters, and cholesteryl esters with squalene or cholesterol, indicating that the cholesteryl ester and squalene compositions may vary independently of other components.

SSL MIMETIC EFFICACY (3%) WHEN APPLIED TOPICALLY

In the first study, the SSL mimetic increased barrier recovery statistically significantly ($p < 0.001$) better than 3% OO, 3% CCT, and the vehicle 60 min posttest article application. These increases were amplified with the inclusion of ceramide 2 (C2), which provided the greatest amplification compared to the other skin-lipid-like emollients (Figure 1).

In the second study, the test article containing the SSL mimetic produced statistically significantly ($p < 0.05$) higher percent changes in skin hydration than all other test articles. The addition of C2 seemed to act synergistically when combined with the SSL mimetic (Figure 2).

In the third study, the SSL mimetic produced statistically significantly ($p < 0.05$) higher percent changes in skin hydration than 3% petrolatum after 1 and 2 weeks of test article use, and following a 1-week regression. Additionally, the SSL mimetic produced statistically significantly ($p < 0.05$) larger decreases in transepidermal water loss (TEWL; an indication of improvement in skin barrier function) after 1 and 2 weeks, and following a 1-week regression (Figure 3). Also, 1 h after application, both products statistically equivalently

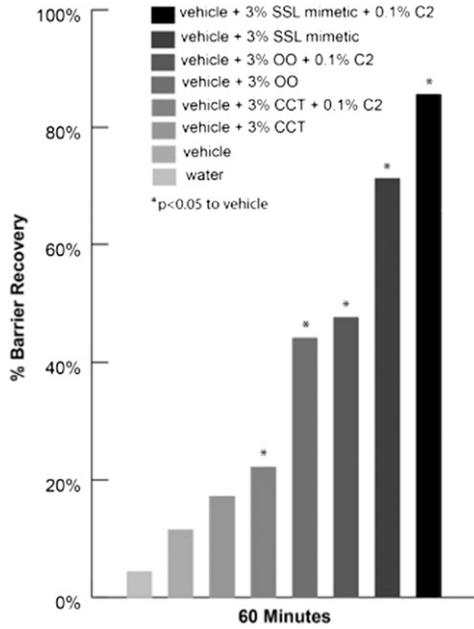


Figure 1. Evaluation of barrier recovery (i.e., reduction in TEWL as compared to the same test site after acetone treatment but prior to test article treatment) 60 min posttest article application.

increased skin hydration (44.1% for the SSL mimetic and 48.8% for petrolatum) and decreased TEWL (-8.2% for the SSL mimetic and -7.1% for petrolatum). This demonstrates the difference between short- and long-term hydration and barrier function effects between the SSL mimetic and an occlusive ingredient such as petrolatum.

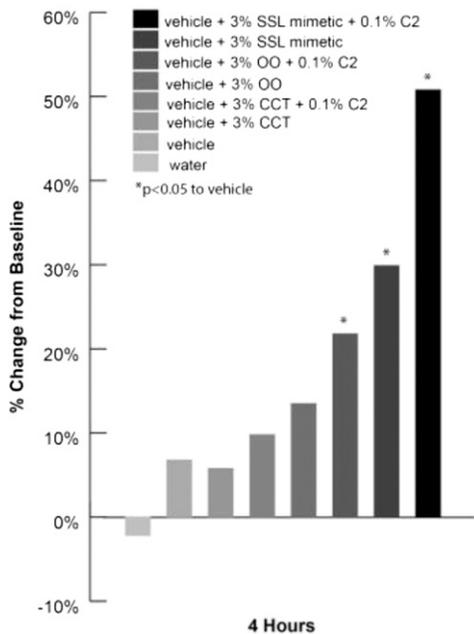


Figure 2. Evaluation of short-term skin hydration 4 h posttest article application.

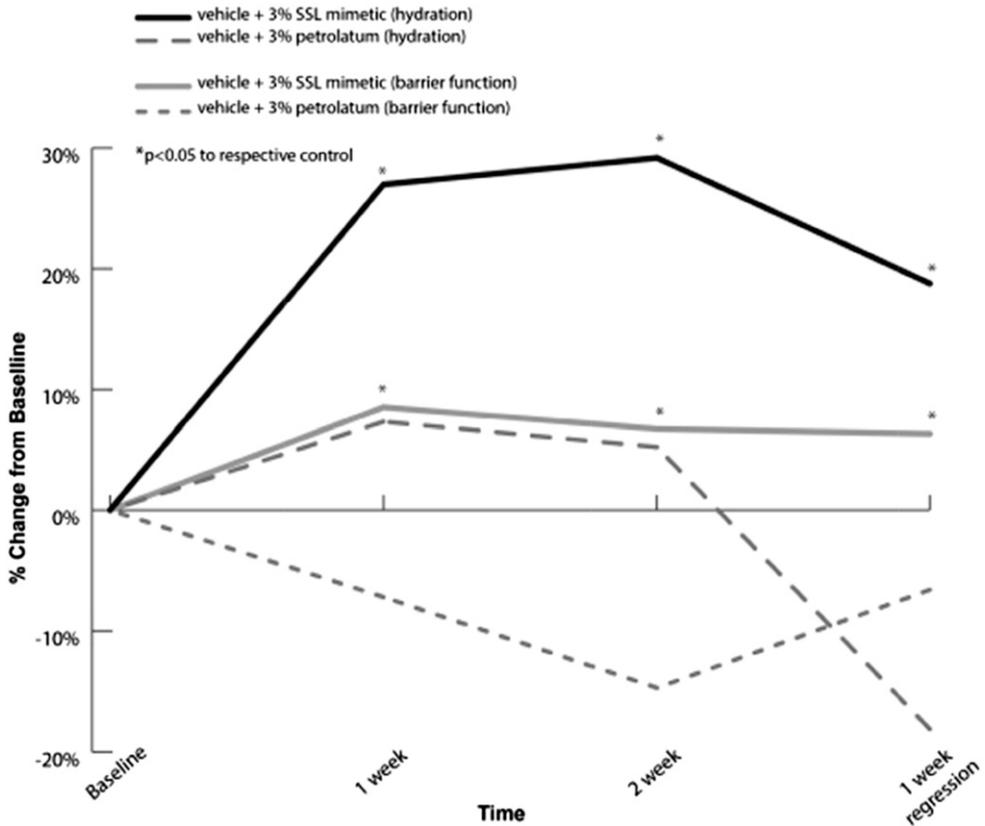


Figure 3. Evaluation of long-term skin hydration and skin barrier function after 2 weeks of test article use followed by 1 week of regression (no test article used).

In the final study, the SSL mimetic produced statistically significant ($p < 0.001$) higher percent changes in skin hydration, elasticity, and firmness than the vehicle (Figure 4).

DISCUSSION

The present research indicated that, overall, the SSL composition is consistent in a controlled population of 22-year-old females. Furthermore, there were strong correlations between the various SSL components, with the exception of squalene and cholesteryl esters. The concentration of FFA demonstrated the greatest variance between subjects and within multiple samples taken from the same subject. The variation in FFA may be due to the differences in the microflora of the subjects (16). When FFAs were combined with glycerides, the coefficient of variation decreased greatly between subjects and between samples taken from the same subject. The variation of this study's results compared to previously published work could indicate that the collection methods, geographic location (10), gender, and age specificity contribute to the distribution or collection of different lipid components on the skin surface. Additionally, factors such as race, hormone-containing birth control, and oily skin did not appear to affect SSL composition (unpublished data collected by Floratech). However, these factors could affect whether or not individuals are prone to acne or the total amount of SSLs on the skin.

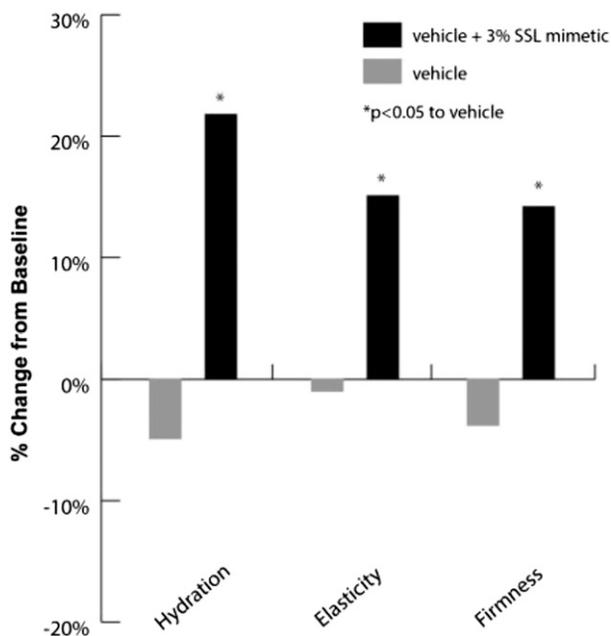


Figure 4. Evaluation of skin hydration, elasticity, and firmness after 1 week of test article use.

Pappas *et al.* (13) explored the possible variation of lipid production and composition based on ethnicities and concluded that composition, particularly wax esters, and lipid output may be affected by ethnicity; however, they did not limit the study to a particular age group. Focusing on a very specific age group, as was done in this research, allowed a less variable comparison since age also affects skin lipid composition (15). Additionally, wax esters play a role in barrier function and hydration (21), so understanding the complex relationship between each of the SSL components could also provide insight into the maintenance of healthy skin.

It is known that diet is a contributing factor in lipid metabolism in skin (22). The high variation of squalene among the subjects in this study, particularly with the unique lipid distribution from vegans and vegetarians, while not significant in number, could potentially be explained by the mechanism of squalene and triglyceride synthesis on a glandular level. It has been shown that acetate directs lipogenesis toward the production of squalene at the expense of triglycerides (23). Similarly, triglyceride synthesis is heavily influenced by the amount of glycogen available to the sebaceous glands (24). A diet low in fat could contribute to this observation; however, more research is needed to prove these mechanisms are significant from a dietary standpoint.

When an SSL mimetic of young, healthy skin is applied topically to the skin, it can impart many of the benefits attributed to the skin's natural SSLs. The mimetic provided better short-term barrier recovery than other botanicals with skin-lipid-like attributes and did not negatively impact long-term barrier maintenance, as was seen with an occlusive ingredient like petrolatum. Similarly, the SSL mimetic provided short- and long-term hydration and limited regression upon discontinuation of product use. It also increased viscoelasticity and hydration in aged skin, demonstrating the importance of replenishing SSLs on skin that has lower quantities due to age.

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