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BIOMEDICAL ANALYSIS OF COSMETIC FORMULATIONS AFFECTING EPIDERMAL REGENERATION LIPID BARRIER AND PH BALANCE

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ABSTRACT
A cosmetic formula is a complicated, multicomponent system that interacts with the epidermis and affects cell turnover, lipids organization, and skin surface acidity. It is known that the stratum corneum lipid matrix and pH physiological gradient control barrier function, water content, and resistance to environmental factors. In this research, a secondary analysis of the Cosmetic and Skin Barrier Biomarker Dataset, cited as available on Kaggle, was used to assess formulation-associated changes in epidermal structure, barrier function, and pH. The dataset compares vehicle control, 0.25% retinol, 5% niacinamide, and their combination over 10 weeks. Variables include TEWL, skin hydration, surface pH, epidermal thickness, and stratum corneum lipid and protein content. No new clinical trial or laboratory measurements were conducted for this analysis. Statistical analysis employed repeated-measures analysis of variance and paired comparison testing using SPSS, with significance set at $p<0.05$. Baseline and follow-up data were examined at predefined intervals over 10 weeks to evaluate formulation-associated changes in biophysical and biochemical skin parameters. Results demonstrated measurable improvements in skin barrier and hydration. TEWL decreased from 14.6 to 8.5 g/m²/h, hydration increased from 37.9 to 60.3 AU, and surface pH decreased from 5.9 to 5.0. Epidermal thickness increased by 8.6 µm, indicating a pronounced treatment- associated structural response with the combined formulation. These dataset findings suggest potential benefits of the evaluated cosmetic formulations in terms of the strengthening of the lipid barrier, regulation of pH levels, and renewal of the epidermis.

Keywords: Epidermal Regeneration, Lipid Barrier Function, Skin PH Homeostasis, Cosmetic Formulation, Transepidermal Water Loss, Stratum Corneum Integrity.

1. Introduction

The primary barrier separating the human body from the outside world is the epidermis, and tissue integrity and epidermal renewal are guaranteed by keratinocyte differentiation and proliferation [1]. Topical repeated application of cosmetic formulations affects the physical, functional, and visual state of facial skin by changing the rate of epidermal turnover, the organization of the barrier, the level of skin moisture, and other parameters [2]. As such, systematic assessment of formulation-induced biological responses is crucial to understand the impact of formulation on epidermal structure-function. The main barrier function of the epidermis is accomplished by the stratum corneum, which is an organized arrangement of corneocytes surrounded by a matrix of intercellular lipids. The balanced ratio of ceramides, cholesterol, and free fatty acids plays a crucial role in maintaining effective skin barrier function [3]. TEWL is an important indicator of barrier integrity, and a low TEWL is usually associated with better barrier function and hence is a useful instrumental parameter to assess cosmetic effects [4]. Barrier-supportive cosmetic components help to enhance the barrier function and hydrotropism of the stratum corneum; ceramides are especially crucial for the maintenance of intercellular lipid organization and water retention [5]. Properly hydrated skin stays smooth, flexible, and resilient, helping it preserve moisture while providing an effective protective barrier against external factors. Craniometry is a quantitative measurement of the hydration of the stratum corneum, and thus is used for determination of formulation-associated improvements [6].

The natural acidity of the skin surface is important for epidermal homeostasis, antimicrobial activity, lipid-processing enzyme activity and barrier function [7]. The pH of the skin surface affects the activity of certain enzymes involved in lipid organization and epidermal differentiation; therefore, cosmetics should maintain a physiological acidic pH to ensure the normal functioning of processes related to the skin barrier [8]. Cosmetic ingredients have the ability to change epidermal properties in several ways, such as moisturization, occlusion, lipid replacement and change of epidermal properties. Therefore, their biological effects should be systematically assessed and quantified by combining complementary biophysical, imaging and biochemical measurements [9]. Topical exposure causes measurable changes in the hydration, barrier function, pH, and structural properties of the epidermis that occur repeatedly. These formulation-related responses can be objectively assessed through instrumental measurements, providing a reliable data on changes in skin properties and performance [10]. The biochemical evaluation of the components and proteins of the stratum corneum yields other information related to the epidermal condition and sheds light on functional changes, which are assessed by biophysical methods [11].

In conjunction with quantitative hydration, TEWL and pH data, high-resolution skin imaging characterizes the surface and epidermal properties of skin [12]. A combination of the structural, biochemical, and biophysical parameters therefore yields better evidence for the ability to distinguish measurable biological effects from cosmetic claims and assess the performance of formulations and skin compatibility [13].

There is great variation among individual skin properties and their reactions to topical formulations. Formulation effects over time are better evaluated with repeated-measures designs, which enable within-subject comparisons and minimize inter-individual variability [14]. Evaluation at several time points after application helps determine the longevity and temporal development of TEWL, hydration, pH, and epidermal changes [15]. A correlation of barrier measurements with hydration, pH, imaging, and biochemical parameters is useful in determining potential relationships between formulation exposure and epidermal responses [16]. This systematic biomedical characterization is used to guide the formulation development of cosmetics on an evidence-based measures of epidermal renewal, barrier competence, hydration, pH homeostasis, skin performance, and formulation compatibility [17]. Individual differences in skin conditions, short assessment periods, and the use of non-invasive biophysical techniques in TEWL, hydration, pH, imaging, and biochemical markers do not fully reflect the complicated lipid arrangement and cellular processes associated with epidermal regeneration. To address these limitations, repeated-measures research, standardized application protocols, multiple assessment times, and measurements of TEWL, hydration, pH, imaging, and biochemical markers are combined. Correlating complementary variables allows the impact of the cosmetic formulation on skin barrier function and regeneration to be assessed in greater detail.

1.1 Research Aim and Key contributions

Research aims to examine the effects of cosmetic products on epidermal regeneration, skin barrier properties, lipid status, and pH maintenance in adult facial skin using standardized biophysical, imaging, and biochemical techniques. Moreover, research analyses the dynamic changes of hydration, TEWL, pH, and biochemical indices following the application of formulations. Key contributions of this research are:

- TEWL, hydration, skin surface pH, imaging, and biochemical measures are combined to evaluate the effects of a cosmetic formulation in comprehensive detail.
- Changes in the epidermal structure and the parameters associated with skin regeneration are assessed through high-quality imaging and analysis of biochemistry.
- Using TEWL, hydration, and lipid parameters, the changes in barrier integrity are evaluated after the cosmetic application has been done.
- Changes in skin surface pH and the influence of those changes on the maintenance of the skin barrier are determined.
- Temporal measurements and statistical correlations are implemented to establish a connection between cosmetic formulation contact and important epidermal effects.

Research is organized into 5 sections: Section 1 presents research background, rationalization, aims, and contribution. Section 2 reviews the related works and previous research relevant of the research. Section 3 describes the materials and methods, including formulation application, participants, and biomedical assessment procedures. Section 4 presents and discusses the experimental results and findings. Section 5 concludes research by outlining its implications, limitations, and directions for future research.

2. Related Works

Previous researches have investigated cosmetic ingredients, skin-barrier function, hydration, lipid organization, epidermal responses, imaging techniques, and pH monitoring using a range of experimental and clinical approaches.

2.1 Cosmetic Ingredients and Skin Barrier Protection

Cosmetic ingredients have been investigated for their capacity to protect and restore skin-barrier function. Nine cosmetic ingredients were examined in terms of their effect on barrier-related genes, cytokines, vascular endothelial growth factor, proliferation, and differentiation on SDS-damaged HaCaT cells [18]. Oat extract exerted a positive influence on barrier-related and inflammatory molecules, but due to the in vitro research design, its clinical significance was not possible to establish. Starch-based cosmetic formulation enriched with Spirulina maxima was assessed by means of rheological analysis, sensory testing, reflectance confocal microscopy, TEWL, hydration and hair tensile tests [19]. TEWL and hair protection were decreased, but due to the short-term research duration, no conclusion on long-term effects could be reached. Another cosmetic formulation was tested in 60 women patients undergoing chemotherapy or radiotherapy treatments [20]. Epidermal hydration level increased; however, a relatively small number of patients and lack of long-term evaluation limit the validity of these results.

2.2 Stratum Corneum Lipid Organization and Hydration

Interdependence of stratum corneum components, hydration and barrier properties has been researched by means of structural and physicochemical analysis. An analysis of glucose and sodium chloride mixture was carried out with the help of solid-state nuclear magnetic resonance, wide-angle X-ray diffraction, and sorption microbalance techniques and allowed to research stratum corneum activity [21]. Mixture decreased the deliquescence relative humidity and increased the mobility of lipids and proteins, but the research was confined to the narrow selection of chemical components without clinical verification. Temperature and hydration dependent phase transition of lipids was researched by permeability researches, infrared spectroscopy and atomic force microscopy [22].

Environmental changes affected the lipid arrangement and compound mobility, however, isolation of stratum corneum models decreased the possibility of generalization for human skin. Similarly, urocanic acid and glycerol were demonstrated to have the different effect on the cohesion of lipids, hydration, and permeability [23]. However, isolation of lipid models and limited clinical verification made the research less applicable.

2.3 Biophysical Assessment of Skin Hydration and Barrier Function

In recent years, non-invasive biophysical approaches have found application in estimating changes in hydration and barrier function. Diffuse reflectance spectroscopy was compared to bioelectrical capacitance in skin hydration estimation in a range of wavelengths from 400 to 1000 nm [24]. A good correlation was obtained, which proved the possibility of using optical estimation methods; however, the impact of external factors and insufficient clinical validation could not be overlooked. The influence of various moisturizers and liquid silicone gel was estimated in 40 volunteers within four hours by means of TEWL, hydration and microcirculation parameters [25]. Various responses were noted, but a short observation period did not allow analyzing long-term effects. An infant moisturizer was tested on babies with either dry or normal skin via moisture and parental questionnaires [26]. Positive results were stated; however, parental evaluation and few objective criteria restricted the completeness of the research. To explore retinol tolerance mechanisms and their effects on skin barrier changes [27]. A 28-day longitudinal evaluation was performed using skin phenotypic measurements, metagenomic sequencing, and metabolomic analysis among retinol-tolerant and intolerant participants. Results showed dynamic microbial and metabolic alterations related to barrier recovery. However, limited participants and short duration reduced clinical applicability.

2.4 Skin Surface pH Monitoring and Homeostasis

Skin surface pH is an important parameter of epidermal homeostasis and has been researched using biological and technological methods. Stratum corneum ceramide composition and filaggrin mutations were tested as biomarkers of atopic dermatitis exacerbation during remission [28]. The shortening of the carbon chain in certain ceramide groups was related to future exacerbation; however, the validation of biomarkers was not sufficient for its broad application. A formulation of Tara gum with Brazilian berries was tested in 10 volunteers through rheological, sensory, clinical, TEWL, hydration, and reflectance confocal microscopy research [29]. It improved hydration, lowered TEWL, and exhibited film formation property; however, the number of participants and research duration did not allow generalization. A sensor with screen-printed polyaniline pH electrode and Ag/AgCl reference electrode allowed to measure skin surface pH reliably and reversibly [30]. However, the necessity of direct contact with the aqueous electrolyte was a restriction for its application.

2.5 Research Gap

Several researches have been carried out to investigate the effect of cosmetic components on skin barrier biomarkers and inflammatory response, but these have often used in vitro approaches, reducing their applicability to real human skin [18]. Several others have assessed cosmetics by employing TEWL, skin hydration, imaging, and biophysical parameters associated with them; however, small sample sizes and evaluation times reduced their reliability [19]. Stratum corneum lipid organization and arrangement have been investigated through other research, which have also contributed in understanding the skin barrier mechanism, but due to isolated approach and lack of in vivo validation, they have been less clinically relevant [23]. Skin pH has been monitored through researches, which showed accurate sensing but limited applicability due to need of direct contact with aqueous electrolyte [30]. The current secondary analysis examines formulation groups, repeated measurements, and reported values of TEWL, hydration, pH, imaging, and biochemical markers to compare formulation-associated patterns; it does not independently resolve limitations of clinical validation.

3. MATERIALS AND METHODS

The methodology describes secondary analysis of an existing dataset, including formulation groups, longitudinal skin parameters, and statistical comparisons. No participants were recruited and no formulations, instrumental measurements, or biological sampling were performed by the authors for this analysis. Figure 1 is retained as a schematic of the study structure described in the manuscript, rather than a clinical trial newly conducted by the authors.

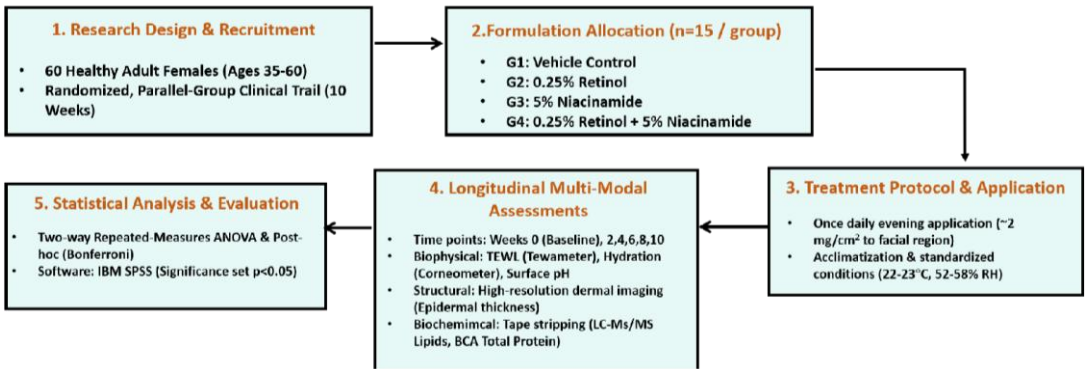


Figure 1: Study Workflow Represented in the Secondary Dataset

3.1 Research Design and Data Source

This study uses secondary data identified in the revised manuscript as the Cosmetic and Skin Barrier Biomarker Dataset, available on Kaggle:
https://www.kaggle.com/datasets/datresearch/cosmetic-and-skin-barrier-biomarker-dataset
The analysis covers 60 longitudinal records in four formulation groups (n = 15 per group): vehicle control (G1), 0.25% retinol (G2), 5% niacinamide (G3), and their combination (G4). The reported assessment times are baseline and Weeks 2, 4, 6, 8, and 10. Variables include TEWL, skin hydration, surface pH, epidermal thickness, ceramides, cholesterol, free fatty acids, and total protein. Formulation groups are summarized in Table 1; Figure 2 provides a mechanistic schematic.
The clinical, application, measurement, and sampling procedures described in Sections 3.2-3.6 are retained as methodological context from the supplied manuscript; they were not performed by the present authors. Their correspondence to the original data-collection documentation, including whether records represent observed or synthetic data, requires verification. Demographic and protocol descriptions must therefore not be interpreted as independently verified clinical provenance.

Table 1: Treatment Groups and Formulation Composition Used for the 10-Week Intervention

Group	Formulation	Active Ingredient(s)	Application Site	Duration	Participants (n)
G1	Vehicle control	No active ingredient			
G2	Retinol formulation	0.25% Retinol			
G3	Niacinamide formulation	5% Niacinamide	Face	10 weeks	15
G4	Combination formulation	0.25% Retinol + 5% Niacinamide			

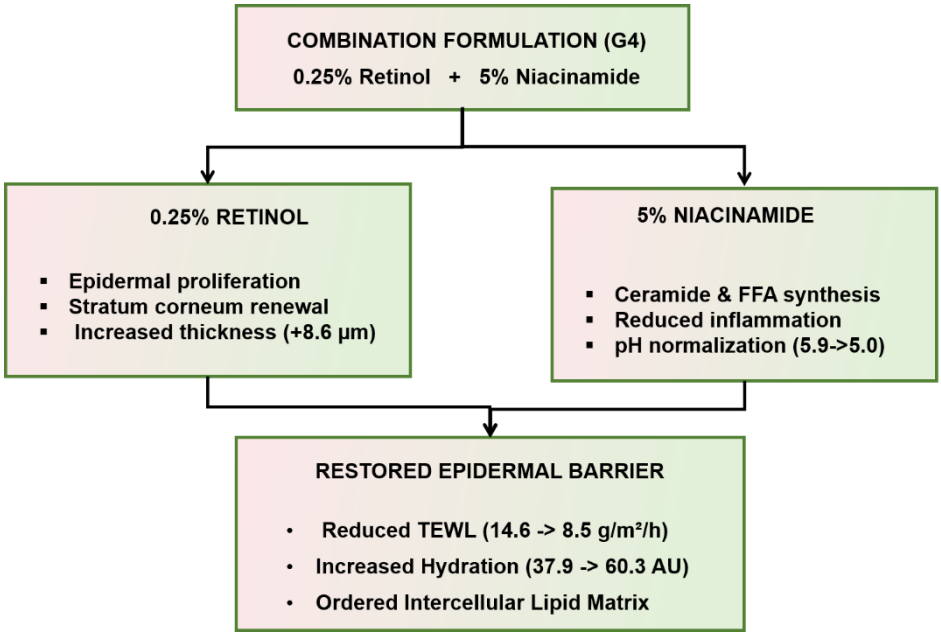


Figure 2: Synergistic Mechanism of Action for the Combination Formulation (G4)

3.2. Cosmetic Formulations and Application Protocol

The topical cosmetic formulations included: a vehicle control (G1), formulation of 0.25% retinol (G2), formulation of 5% niacinamide (G3) and formulation of 0.25% retinol and 5% niacinamide (G4). All the four topical treatments were applied on the basis of the allocated treatment groups for the full period of 10 weeks of intervention. Topical treatment was done once a day in the evening in a dose of about 2 mg/cm² onto the designated facial treatment area, which was full face area excluding periorbital region. The participants were advised to apply the formulation to the designated face area consistently after cleaning it. They were supposed to be consistent with this process all through the research.
It was advised that the participants do not use any other skin care products, peeling agent, topical retinoids, or other treatment products on the target area during the intervention phase. The participants were allowed to use sunscreen, which had an SPF level of 30 or higher, during daylight hours. The participants were advised not to introduce any other cosmetic products during the research period. Adherence to the treatment plan was monitored using the application diaries of the participants and through the empty treatment product containers.

Vehicle control formulation was used as a comparator for assessing formulation-specific differences in biophysical and biochemical skin parameters. All formulations were kept under controlled storage conditions and applied uniformly throughout the trial period. Concentrations of active ingredients in all formulations remained the same throughout the duration of the research. Composition of the four formulations and important INCI ingredients are shown in Table 2.

Table 2: Composition of the Experimental Cosmetic Formulations Used During the 10-Week Intervention

Formulation	Active Ingredient(s)	Concentration	Key INCI Ingredients
Vehicle Control (G1)	None	0%	Aqua, Glycerin, Caprylic/Capric Triglyceride, Cetearyl Alcohol, Glyceryl Stearate Citrate, Squalane, Sodium Hyaluronate, Carbomer, Phenoxyethanol, Ethylhexylglycerin
Retinol Formulation (G2)	Retinol	0.25%	Aqua, Retinol, Coco-Caprylate/Caprate, Glycerin, Cetearyl Alcohol, Steareth-2, Steareth-21, Dimethicone, Squalane, Tocopherol, Sodium Hyaluronate, Carbomer, Phenoxyethanol
Niacinamide Formulation (G3)	Niacinamide	5%	Aqua, Niacinamide, Glycerin, Betaine, Squalane, Ceramide NP, Cholesterol, Cetearyl Alcohol, Glyceryl Stearate Citrate, Sodium Hyaluronate, Carbomer, Phenoxyethanol
Combination Formulation (G4)	Retinol + Niacinamide	0.25% + 5%	Aqua, Niacinamide, Retinol, Coco-Caprylate/Caprate, Glycerin, Cetearyl Alcohol, Squalane, Ceramide NP, Cholesterol, Dimethicone, Tocopherol, Sodium Hyaluronate, Carbomer, Phenoxyethanol

3.3 Research Protocol and Controlled Conditions

The baseline was established prior to applying the cosmetic product (Week 0). The following assessments were carried out at Weeks 2, 4, 6, 8, and 10 to assess the longitudinal changes in skin barrier properties, hydration level, pH value, histological structure of epidermis, and biochemical parameters during the course of intervention. All the assessments were conducted in a laboratory environment with constant temperature 22–23 °C and humidity 52–58%.

Before each evaluation, the subjects attended without facial makeup and were advised against any facial washing or use of skin care or cosmetics for 3 hours prior to the testing. The subjects were also informed not to apply the test formula that was prescribed for them before each evaluation session. There was a 30-minute period allocated for acclimatization prior to the instrument testing, to decrease the effect of environmental factors on the skin parameters. Where feasible, assessments were conducted at approximately the same time of day for each participant across research visits.

The same facial measurements and samplings were employed at all measurement intervals. The assessments of TEWL, skin hydration, surface pH, epidermal imaging, and stratum corneum samplings were made by means of standardized methods in similar measuring conditions throughout the whole duration of the research. This was done at each visit point to enhance temporal comparability and enable evaluation of formulation-associated changes over the 10-week intervention period.

3.4 Biophysical Skin Assessments

Skin barrier function, hydration, skin surface pH, and epidermal thickness were measured by means of standard non-invasive instrumental technique at baseline (Week 0), Weeks 2, 4, 6, 8, and 10. Testing was carried out 30 minutes after acclimation under controlled room temperature of 22–23 °C and relative humidity of 52–58%. The lateral cheek area was the predefined measurement area, and the mean of three successive readings per parameter was calculated.

Transepidermal Water Loss (TEWL): TEWL measurement was done with the use of Tewameter® TM300 and results were given as g/m²/h. Three measurements of the lateral cheek were done and their average was noted. Reduced TEWL values suggested enhanced function of the skin barrier.

Skin Hydration: Skin hydration was quantified by means of a Corneometer® CM 825 and given in AU. The measurement was done in triplicates at the same spot on the cheek, and the average was taken as the final reading.

Surface pH: The skin surface pH was measured by a Skin-pH-Meter® PH 905. This was done by taking three readings at the preselected area of the cheek and calculating their average. The results were recorded as the pH units, where variations were explained in relation to physiologic mildly acidic skin surface environment.

Epidermal Thickness: The average baseline epidermal thickness was roughly 58.9 ± 4.1 μm for all four treatment group formulations. The changes in epidermal thickness were assessed at Weeks 2, 4, 6, 8, and 10, and the results were deemed to be related to the remodeling of the structure due to the intervention, and not as a direct measurement of epidermal regeneration.

Biophysical measurements were then correlated with analysis of stratum corneum lipids and proteins to determine any changes in skin state, barrier function, hydration and pH balance due to the formulation.

3.5 Stratum Corneum Lipid Sampling and Analysis

The stratum corneum lipids were obtained from the defined lateral cheek site via tape stripping at Weeks 0, 2, 4, 6, 8, and 10. Three successive adhesive tapes were attached to the designated skin area under the same pressure and contact period. The adhesive tapes were transferred to individual tubes compatible with lipids and stored at −80 °C.

Lipid extraction was achieved through an organic solvent-based method, after which centrifugation, nitrogen drying, and reconstitution in the proper solvent were carried out. Ceramides, cholesterol, and free fatty acids were quantitatively determined using liquid chromatography–mass spectrometry (LC-MS/MS). The calibration standards and the suitable internal standards were used for the quantitative analysis of lipids, and the lipids were expressed in μg/cm².

Variations in ceramide, cholesterol, and free fatty acid content were correlated between formulation treatment groups and various times of assessment. This was supplemented with a combined analysis of the results obtained for TEWL, skin hydration, surface pH, epidermal thickness, and stratum corneum protein to assess skin barrier performance and epidermal status.

3.6 Stratum Corneum Protein Sampling and Biochemical Analysis

Stratum corneum samples were obtained from the pre-designated lateral cheek area via standardized tape stripping at baseline (Week 0), and Weeks 2, 4, 6, 8, and 10. Three sequential tape strippings were placed on the same area with the same pressure and contact time. The tape samples were then stored separately in sterile microcentrifuge tubes at −80°C.

In protein isolation, each strip was placed into 500 μL PBS and was gently shaken for 15 minutes and then centrifuged for 10 minutes at about 10,000 xg. The collected supernatant was analyzed. Protein content was quantified using BCA colorimetry at a wavelength of 562 nm in a microplate spectrophotometer. Protein concentration was calculated from a bovine serum albumin (BSA) calibration curve and expressed as μg/strip.

Protein levels of total stratum corneum were analyzed for comparison among different formulation groups at each assessment period. Protein results were evaluated in conjunction with TEWL, skin hydration, skin pH, epidermal thickness, and lipids to give a comprehensive analysis of changes in the epidermal state and skin-barrier function due to formulations.

3.7 Statistical Analysis

All statistical analysis was carried out using the IBM SPSS Statistics version 27. Continuous variables were expressed as mean ± SD. Variations in TEWL, hydration, surface pH, epidermal thickness, and SC lipid and protein characteristics were measured for each of the four formulation groups at six separate measurement times. Two-way repeated measures ANOVA were conducted to measure the effect of treatment group, time, and group-time interaction. When statistically significant differences were observed, Bonferroni-corrected post hoc tests were done to identify the specific inter-group and intra-group differences. Changes from baseline to post-intervention were also compared using paired tests when necessary. $p < 0.05$ was used for statistical significance, and p-values and effect size were provided in addition to the results. Statistical data were used together to evaluate changes that have occurred in skin structure, skin barrier function, lipid profile, hydration, and surface pH due to formulations.

4. RESULTS

The outcome of the experiment revealed that significant positive changes were observed in the barrier function of the skin, hydration level, skin surface pH, epidermis thickness, stratum corneum lipid profile, and proteins. The statistical test was carried out using IBM SPSS software where repeated measure ANOVA was applied for data analysis. Among the treatments, the combination had the highest effect, followed by niacinamide and retinol, while the vehicle control showed comparatively smaller changes across the evaluated parameters.

4.1 Demographic Characteristics and Group Comparability

The secondary dataset is represented by 60 records across four formulation groups (n = 15 per group). The mean ages varied from 47.1 ± 6.5 years in the vehicle control group to 48.0 ± 6.7 years in the niacinamide group. Baseline TEWL values ranged from 14.5 ± 1.8 to 14.7 ± 1.9 g/m²/h, while skin hydration values ranged from 37.9 ± 4.3 to 38.3 ± 4.5 AU across the four groups. Surface pH was consistent across groups at 5.9 ± 0.2, and baseline epidermal thickness ranged from 58.7 ± 4.1 to 59.0 ± 4.3 μm. The overall baseline characteristics in demographic and skin were similar between the four groups. The overall baseline characteristics of the dataset records are given in Table 3.

Table 3: Demographic and Skin Characteristics of Participants in Descriptive Statistics

Parameter	G1 Control	G2 Retinol	G3 Niacinamide	G4 Retinol + Niacinamide
Participants (n)	15	15	15	15
Age (years)	47.1 ± 6.5	47.8 ± 6.9	48.0 ± 6.7	47.5 ± 7.1
TEWL (g/m²/h)	14.5 ± 1.8	14.7 ± 1.9	14.6 ± 1.7	14.6 ± 1.8
Hydration (AU)	38.3 ± 4.5	38.0 ± 4.2	38.2 ± 4.4	37.9 ± 4.3
Surface pH	5.9 ± 0.2	5.9 ± 0.2	5.9 ± 0.2	5.9 ± 0.2
Epidermal thickness (μm)	58.7 ± 4.1	59.0 ± 4.3	58.8 ± 4.0	58.9 ± 4.2

4.2 Effects of Cosmetic Formulations on TEWL, Skin Hydration, and Surface pH

Progressive changes in biophysical skin parameters were observed over the 10-week intervention period. TEWL decreased from baseline to Week 10 in all four formulation groups, with the smallest reduction observed in the vehicle control group and progressively greater reductions in the active formulation groups. The greatest numerical reduction was observed in G4 (0.25% retinol + 5% niacinamide), consistent with improved skin-barrier function. Skin hydration increased across all groups, with the largest increase observed in G4, followed by G3 and G2. Surface pH decreased from approximately 5.9 at baseline toward a mildly acidic physiological range, with the largest numerical changes observed in G3 and G4. Overall, the biophysical findings indicated improvements in skin-barrier function, hydration, and surface pH regulation during the intervention period. Baseline skin surface diagnostics revealed reduced moisture retention and irregular micro-relief before treatment exposure in Table 4, aligning with initial hydration and TEWL measurements; imaging of the lateral cheek surface demonstrated pronounced epidermal dryness, fine micro-fissures, and irregular surface micro-relief indicative of baseline barrier impairment (TEWL: 14.6 ± 1.8 g/m²/h, Hydration: 37.9 ± 4.3 AU).

Table 4: Changes in TEWL, Skin Hydration, and Surface pH from Baseline to Week 10

Parameter (Mean ± SD)	G1 Control	G2 0.25% Retinol	G3 5% Niacinamide	G4 Retinol + Niacinamide	p (Time)	p (Group × Time)
TEWL (g/m²/h)	14.5 ± 1.8 → 12.0 ± 1.6	14.7 ± 1.9 → 10.5 ± 1.4	14.6 ± 1.7 → 9.8 ± 1.3	14.6 ± 1.8 → 8.5 ± 1.2	<0.001	<0.001
Skin Hydration (AU)	38.3 ± 4.5 → 46.0 ± 4.5	38.0 ± 4.2 → 50.5 ± 4.7	38.2 ± 4.4 → 54.0 ± 4.9	37.9 ± 4.3 → 60.3 ± 5.1	<0.001	<0.001
Surface pH	5.9 ± 0.2 → 5.3 ± 0.2	5.9 ± 0.2 → 5.1 ± 0.2	5.9 ± 0.2 → 5.0 ± 0.2	5.9 ± 0.2 → 5.0 ± 0.2	<0.001	0.002

4.3 Changes in Skin Hydration during the Treatment Period

There was a progressive increase in skin hydration over the 10-week trial for all the four formula groups. In the vehicle control group, there was a moderate increase in skin hydration, while in the retinol and niacinamide groups, there was a considerable improvement. The combination formula (G4) resulted in the highest increase in hydration, increasing from 37.9 ± 4.3 AU to 60.3 ± 5.1 AU from Week 0 to Week 10. There was a significant increase in skin hydration in relation to time, and there was a significant group by time interaction effect. The changes along the longitudinal axis are illustrated in Table 5.

Table 5: Time-Dependent Changes in Skin Hydration Following Cosmetic Formulation Treatment

Time point	G1 Control	G2 0.25% Retinol	G3 5% Niacinamide	G4 Retinol + Niacinamide
Baseline	38.3 ± 4.5	38.0 ± 4.2	38.2 ± 4.4	37.9 ± 4.3
Week 2	39.1 ± 4.4	41.0 ± 4.5	41.8 ± 4.6	43.0 ± 4.7
Week 4	40.0 ± 4.5	43.7 ± 4.6	44.9 ± 4.8	46.6 ± 4.9
Week 6	40.6 ± 4.4	45.8 ± 4.7	47.2 ± 4.9	49.0 ± 5.0
Week 8	41.2 ± 4.6	47.5 ± 4.8	49.3 ± 5.0	51.0 ± 5.1
Week 10	46.0 ± 4.5	50.5 ± 4.7	54.0 ± 4.9	60.3 ± 5.1
Change from baseline	+7.7	+12.5	+15.8	+22.4
Within-group p-value*	<0.001	<0.001	<0.001	<0.001

4.4 Structural Changes in Epidermal Thickness

A gradual increase in epidermal thickness occurred during the 10-week intervention for all the formulation groups. The vehicle control group exhibited a slight structural change while both the retinol and niacinamide formulations elicited higher increase in the thickness. There was a more significant increase in the combination formulation (G4) in epidermal thickness, implying structural remodeling. There was a gradual increase in epidermal thickness from 58.9 µm at the baseline to Week 2, Week 4, Week 6, Week 8, and Week 10. Time-related structural change was significantly evident from the longitudinal study, and there was a significant group by time interaction in relation to the changes in structure. The results are illustrated in Table 6.

Table 6: Longitudinal Changes in Epidermal Thickness across the Four Formulation Groups

Time point	G1 Control	G2 0.25% Retinol	G3 5% Niacinamide	G4 Retinol + Niacinamide
Baseline	58.7 ± 4.1	59.0 ± 4.3	58.8 ± 4.0	58.9 ± 4.2
Week 2	59.2 ± 4.1	60.4 ± 4.3	60.8 ± 4.1	61.5 ± 4.2
Week 4	59.7 ± 4.2	61.6 ± 4.4	62.2 ± 4.2	63.1 ± 4.3
Week 6	60.1 ± 4.2	62.8 ± 4.5	63.5 ± 4.3	64.8 ± 4.4
Week 8	60.5 ± 4.2	64.0 ± 4.5	64.8 ± 4.4	66.2 ± 4.5
Week 10	60.8 ± 4.3	65.1 ± 4.6	66.0 ± 4.5	67.5 ± 4.6

Change from baseline (µm)	+2.1	+6.1	+7.2	+8.6
Within-group p-value*	0.041	<0.001	<0.001	<0.001

4.5 Changes in Stratum Corneum Lipid Composition

Analysis of stratum corneum lipids indicated that there was an overall increase in the number of measured ceramides, cholesterol, and free fatty acids. Changes were lower in the vehicle control group while greater changes were noted in the active formulations. The combined formulation (G4) group displayed the greatest increases in terms of the amounts of the primary lipids. Such results suggest increased levels of measured stratum corneum lipids and could help enhance barrier function. The baseline and Week 10 lipids in the four formulation groups are summarized and G4 showed the highest increases of ceramides +0.65, cholesterol +0.52, FFA +0.39 µg/cm² in Table 7.

Table 7: Baseline-to-Week 10 Changes in Stratum Corneum Lipid Composition

Lipid component (µg/cm²)	G1 Control	G2 0.25% Retinol	G3 5% Niacinamide	G4 Retinol + Niacinamide
Ceramides – Baseline	1.82 ± 0.24	1.80 ± 0.23	1.81 ± 0.22	1.83 ± 0.24
Week 10	1.96 ± 0.25	2.18 ± 0.27	2.31 ± 0.28	2.48 ± 0.30
Change	+0.14	+0.38	+0.50	+0.65
Cholesterol – Baseline	2.41 ± 0.31	2.39 ± 0.30	2.40 ± 0.29	2.42 ± 0.31
Week 10	2.55 ± 0.32	2.72 ± 0.33	2.81 ± 0.34	2.94 ± 0.35
Change	+0.14	+0.33	+0.41	+0.52
Free fatty acids – Baseline	1.36 ± 0.19	1.35 ± 0.18	1.37 ± 0.19	1.36 ± 0.18
Week 10	1.44 ± 0.20	1.58 ± 0.21	1.66 ± 0.22	1.75 ± 0.23
Change	+0.08	+0.23	+0.29	+0.39

4.6 Changes in Total Protein Content

Total protein content had a gradual increase in its value over the 10 weeks of treatment in all the different formulations. Vehicle control group has a modest change in the total protein content compared to retinol and niacinamide formulations which have high changes in total protein content. The combination formulation group (G4) had the highest numerical increase in total protein content at Week 10 from the baseline. This shows that there was an increase in total protein content of stratum corneum and offers biochemical support for the treatment effects. However, total protein content was considered to be just a supporting biochemical parameter, not a direct indicator of epidermal regeneration. The obtained results of G4 showed the highest protein increase, rising from 18.4 ± 2.6 to 24.5 ± 3.2 µg/strip (+6.1 µg/strip) in Table 8.

Table 8: Changes in Total Protein Content During the 10-Week Treatment Period

Time point	G1 Control (µg/strip)	G2 0.25% Retinol (µg/strip)	G3 5% Niacinamide (µg/strip)	G4 Retinol + Niacinamide (µg/strip)
Baseline	18.4 ± 2.6	18.5 ± 2.5	18.3 ± 2.7	18.4 ± 2.6
Week 2	18.7 ± 2.6	19.4 ± 2.6	19.6 ± 2.7	20.0 ± 2.7
Week 4	19.0 ± 2.7	20.2 ± 2.7	20.5 ± 2.8	21.1 ± 2.8
Week 6	19.2 ± 2.7	21.0 ± 2.8	21.4 ± 2.9	22.2 ± 2.9
Week 8	19.5 ± 2.8	21.7 ± 2.9	22.2 ± 3.0	23.3 ± 3.0
Week 10	19.8 ± 2.8	22.4 ± 3.0	23.0 ± 3.1	24.5 ± 3.2
Change from baseline	+1.4	+3.9	+4.7	+6.1
Within-group p-value*	0.038	<0.001	<0.001	<0.001

4.7 Statistical Comparison and Treatment Effects

Analysis of repeated measures indicated a statistically significant effect of time for each of the skin parameters measured during the 10-week study. Statistically significant effects of time were recorded for TEWL, skin hydration, skin surface pH, epidermal thickness, stratum corneum lipids composition, and total protein content ($p < 0.001$). There was also a significant interaction of Group x Time for all skin parameters evaluated. This means that there was a difference in the extent to which each parameter changed among the four groups of formulations. Formulation G4, which was the combination formulation, and gave the most numerical change in each of the skin parameters analyzed, whereas the vehicle control showed comparatively smaller changes. The overall treatment-related changes and corresponding statistical findings are summarized and G4 achieved maximum changes of TEWL −6.1 and hydration +22.4 in Table 9.

Table 9: Statistical Comparison of Treatment Effects across the Four Formulation Groups

Outcome Parameter	G1 Control	G2 Retinol	G3 Niacinamide	G4 Retinol + Niacinamide	p (Time)	p (Group × Time)
TEWL (g/m²/h)	−2.5 ± 0.8	−4.2 ± 0.9	−4.8 ± 1.0	−6.1 ± 1.1	<0.001	<0.001
Skin Hydration (AU)	+7.7 ± 2.1	+12.5 ± 2.4	+15.8 ± 2.7	+22.4 ± 3.1	<0.001	<0.001
Surface pH	−0.1 ± 0.1	−0.8 ± 0.1	−0.9 ± 0.1	−0.9 ± 0.1	<0.001	0.002
Epidermal Thickness (μm)	+2.1 ± 1.0	+6.1 ± 1.4	+7.2 ± 1.5	+8.6 ± 1.7	<0.001	<0.001
Ceramides (μg/cm²)	+0.14 ± 0.07	+0.38 ± 0.10	+0.50 ± 0.11	+0.65 ± 0.13	<0.001	<0.001
Cholesterol (μg/cm²)	+0.14 ± 0.06	+0.33 ± 0.08	+0.41 ± 0.09	+0.52 ± 0.10	<0.001	0.001
Free Fatty Acids (μg/cm²)	+0.08 ± 0.04	+0.23 ± 0.06	+0.29 ± 0.07	+0.39 ± 0.08	<0.001	<0.001
Total Protein (μg/strip)	+1.4 ± 0.6	+3.9 ± 0.9	+4.7 ± 1.0	+6.1 ± 1.2	<0.001	<0.001

4.8 Overall Comparative Performance of the Four Formulations

Overall comparison of the evaluated outcomes showed a greater treatment-associated response with the combined retinol and niacinamide formulation (G4) than with the individual active formulations and vehicle control. G4 demonstrated the largest numerical improvement in TEWL, skin hydration, epidermal thickness, stratum corneum lipid parameters, and total protein content. The niacinamide formulation (G3) generally produced the next greatest response, followed by the retinol formulation (G2), while the vehicle control (G1) showed comparatively smaller changes. Surface pH decreased toward a mildly acidic physiological range in all groups, with greater reductions observed in the active formulations. Overall, the combined formulation showed the most pronounced numerical changes across the assessed skin barrier, hydration, structural, and biochemical parameters, G4 achieved highest lipid increases of ceramides +0.65, cholesterol +0.52, FFA +0.39, which is presented in Table 10.

Table 10: Overall Comparative Performance of the Four Cosmetic Formulations

Outcome Parameter	G1 Control	G2 0.25% Retinol	G3 5% Niacinamide	G4 Retinol + Niacinamide
TEWL change (g/m²/h)	−2.5 ± 0.8	−4.2 ± 0.9	−4.8 ± 1.0	−6.1 ± 1.1
Skin hydration change (AU)	+7.7 ± 2.1	+12.5 ± 2.4	+15.8 ± 2.7	+22.4 ± 3.1
Surface pH change	−0.6 ± 0.1	−0.8 ± 0.1	−0.9 ± 0.1	−0.9 ± 0.1
Epidermal thickness change (μm)	+2.1 ± 1.0	+6.1 ± 1.4	+7.2 ± 1.5	+8.6 ± 1.7
Ceramide change (μg/cm²)	+0.14 ± 0.07	+0.38 ± 0.10	+0.50 ± 0.11	+0.65 ± 0.13
Cholesterol change (μg/cm²)	+0.14 ± 0.06	+0.33 ± 0.08	+0.41 ± 0.09	+0.52 ± 0.10
Free fatty acid change (μg/cm²)	+0.08 ± 0.04	+0.23 ± 0.06	+0.29 ± 0.07	+0.39 ± 0.08
Total protein change (μg/strip)	+1.4 ± 0.6	+3.9 ± 0.9	+4.7 ± 1.0	+6.1 ± 1.2

4.9 DISCUSSION

The findings suggest that the tested cosmetic formulations positively influenced skin barrier function, hydration, pH, and epidermal regeneration. Among the groups, the combined retinol and niacinamide formulation showed the most consistent improvement across the measured parameters. It indicates that combining both active ingredients provides complementary benefits for overall skin condition.

4.9.1 Existing Limitations and Interpretation of Key Findings

Previous research has highlighted several limitations, including individual and site-specific variability in skin pH measurements [31]. Evidence extraction was limited by insufficient human validation, short-term assessment, and a lack of information on long-term efficacy, safety, and clinical applicability [32]. Further restrictions in research on γ-PGA were related to limited clinical validation, long-term results, and studies on humans [33]. Niacinamide researches were performed using isolated stratum corneum model only and therefore their clinical relevance became low [34]. Also, other results were achieved due to in vitro research and need validation on human skin [35].

The current secondary analysis compares reported TEWL, hydration, pH, imaging-related, and biochemical outcomes across formulation groups. It does not provide new human experiments or independent clinical validation. The study structure and reported outcomes after 10 weeks in all the

4 experimental groups are illustrated in Figure 3. The formulation performs better synergistically with transepidermal water loss reduction of $-6.1\ g/m^2/h$ and an epidermal thickness increase of $+8.6\ \mu m$.

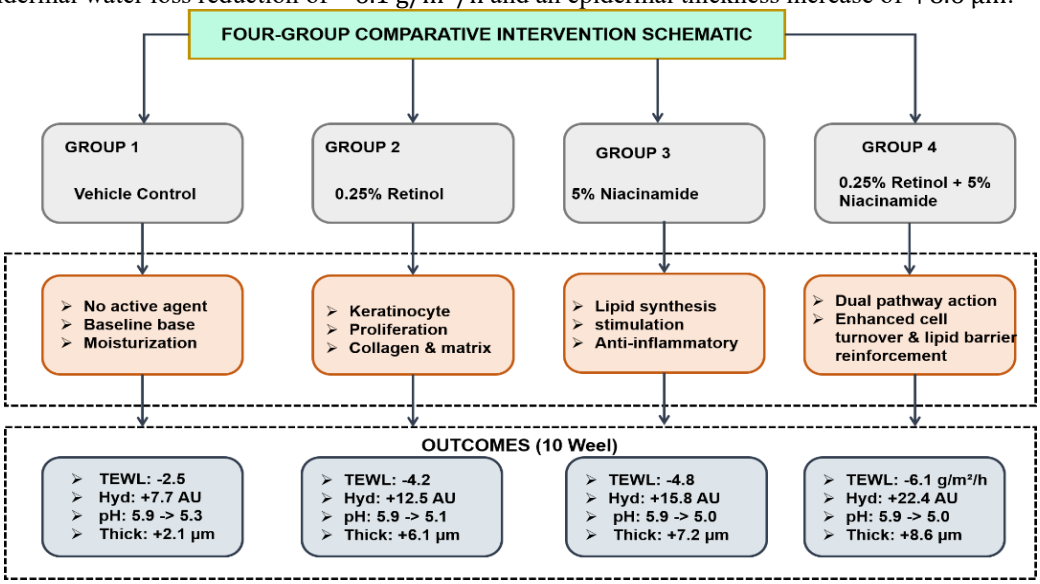


Figure 3: Four-Group Comparative Intervention Schematic and Outcomes

The findings demonstrate that the combined retinol and niacinamide formulation (G4) produced the most pronounced improvements across the evaluated parameters. TEWL is decreased and skin hydration is increased, indicating improved moisture retention and barrier-associated function. G4 also showed the greatest increases in ceramides, cholesterol, free fatty acids, epidermal thickness, and total protein, while surface pH decreased toward an acidic physiological range. These concurrent changes indicate that the combined formulation influences multiple aspects of epidermal condition, including barrier-associated lipid composition, hydration, structural characteristics, and surface homeostasis. The greater response observed with G4 compared with the individual formulations suggests complementary contributions from retinol and niacinamide. However, because the tested formulations contain multiple components, the findings should not be interpreted as definitive evidence of pharmacological synergy. Furthermore, the increase in epidermal thickness should be considered as an evidence of structural change rather than direct evidence of epidermal regeneration because cellular regeneration was not directly assessed.

4.9.2 Practical Implications for Topical Formulation Development

The findings have practical implications for the development of topical formulations targeting multiple aspects of skin condition. The stronger overall response observed with G4 supports the potential value of combining complementary active ingredients rather than evaluating barrier function or hydration through a single endpoint. The simultaneous assessment of TEWL, hydration, pH, epidermal thickness, stratum corneum lipids, and protein provides a broader characterization of formulation-related skin responses. Further investigations should include larger and more diverse populations, extended follow-up periods, dose-response assessments, and molecular and cellular measurements to clarify the mechanisms of epidermal regeneration, lipogenesis, and barrier recovery and to determine whether the observed effects are sustained over time.

5. CONCLUSION

Secondary analysis of the reported dataset suggests that the combination of 0.25% retinol + 5% niacinamide had the best improvement in the properties of facial skin in 10 weeks. The TEWL was reduced from 14.6 to 8.5 g/m2/hr, while hydration was increased from 37.9 to 60.3 AU and surface pH from 5.9 to 5.0. There is an increase in epidermal thickness of 8.6 µm along with increased amounts of ceramide, cholesterol, free fatty acid, and proteins. In comparison to other individual formulations and vehicle control, the combination formula had the maximum improvement effects associated with the treatment. These findings support its potential for improving skin barrier function, hydration, pH balance, and structural skin characteristics.

However, it also has certain drawbacks, which include its fairly limited sample size, 10-week follow-up period, and reliance on secondary data with unverified primary collection procedures. Further research on this topic should be conducted to confirm the results presented in this research. Future research can prioritize studies involving larger and more diverse populations, along with extended follow-up periods to better evaluate long-term outcomes. It can help in understanding whether or not the positive effect of these treatments is permanent. Moreover, future research needs to conduct molecular and cellular assessments of epidermal regeneration, lipogenesis, and barrier recovery. Another area to consider in future research is dose-response studies, as well as mechanistic assessments of retinol and niacinamide at various concentrations, along with mechanistic analyses, it may further clarify their individual and combined effects on skin barrier function and structural remodeling.

Appendix

Abbreviation	Full form
TEWL	Transepidermal water loss
FFA	Free fatty acids
pH	Potential of Hydrogen
HaCaT	Human keratinocyte cell line
VEGF	Vascular Endothelial Growth Factor
NMR	Nuclear Magnetic Resonance
RH	Relative Humidity
FLG	Filaggrin
DRS	Diffuse reflectance spectroscopy
XSS	Xerosis Severity Scale
IGA	Investigator's Global Assessment
Ag	Silver
AgCl	Silver Chloride
$\gamma - PGA$	Gamma − Polyglutamic Acid.
ANOVA	Analysis of variance
PBS	phosphate − buffered saline
SPSS	Statistical Package for the Social Sciences
BCA	bicinchoninic acid
BSA	bovine serum albumin
PBS	phosphate − buffered saline
SD	standard deviation
AU	arbitrary units
SDS	Sodium Dodecyl Sulfate

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