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Natural Moisturizing Factor-Enriched Formulations Compared to a Ceramide-Based Cream

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ABSTRACT

Background: We aimed to investigate the effects of 2 ceramide plus natural moisturizing factor-enriched formulations compared to a ceramide-based cream on skin moisturization.

Methods: Two double-blinded comparative studies were conducted, which enrolled 35 (n=29 females, n=6 males) and 33 (n=21 females, n=12 males) participants, respectively. Participants applied ceramide plus natural moisturizer cream or ceramide-based cream (study 1) or applied ceramide plus natural moisturizing factor lotion or ceramide-based cream (study 2) to each of their lower legs for 10 days with a 5-day regression period (no moisturizer applied). Skin hydration by corneometry after bilateral application was conducted once daily for each leg in both groups.

Results: An increase in corneometer units vs baseline for the ceramide plus natural moisturizing factor-enriched cream and natural moisturizing factor-enriched lotion were greater than the increase vs baseline for the ceramide-based cream at days 10 and 15; with an overall statistical significance in favor of the ceramide plus natural moisturizing factor-enriched formulations at day 10.

Conclusions: The marked improvement in skin moisturization following utilization of the ceramide plus natural moisturizing factor-enriched cream and lotion compared to the ceramide-based cream can be attributed to the inherent properties of the natural moisturizing factors. These properties are known to maintain the humectancy and intercellular lipid membrane of the stratum corneum, which directly improves the permeability barrier function of human skin in reducing transepidermal water loss.

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INTRODUCTION

The skin is a complex barrier composed of tightly packed junctions consisting of 3 parts: stratified epidermis, dermis, and subcutaneous tissue.[1] A primary function of the skin is to maintain a physical barrier against external exposures, preventing entry of harmful substances and maintaining physiologic water content.[1] The permeability barrier function of the epidermis prevents excessive fluid gain or loss from the body occurring within the stratum corneum (SC), the outer portion of the epidermis, which is collectively active in maintaining multiple skin barrier functions necessary to sustain healthy skin.[2] When the skin is compromised by exogenous and/or external environmental exposures, permeability barrier function is impaired. This leads to increased transepidermal water loss (TEWL), which predisposes the skin to adverse sequelae of xerosis; microfissuring; decreased resiliency; natural elastic recoil, scaling, and hyperkeratosis; and an increased risk of microbial infections.[2-4] The water content inside the SC is essential to maintaining the structural and functional integrity of the skin and the multiple functions of the skin barrier.[3,5]

The SC usually is described as a brick-and-mortar complex filled with keratin cells and a lipid matrix, which sounds fixed in its structure, but is dynamic, responds to exposures, and corrects itself with innate repair mechanisms.[2,6,7] Specific enzymes in the epidermis act on phospholipids to synthesize ceramides, free fatty acids, and cholesterol in a physiologic ratio of relative concentrations, thus regulating the permeability and structural functions of the SC.[8,9] Ceramides are the predominant lipid of the SC, making up approximately 50% of the intracellular content by mass.[10] However, the SC contains additional key elements associated with barrier functionality, including urea, lactic acid, and filaggrin-derived amino acids and amino acid metabolites (ie, pyrrolidone carboxylic acid [PCA] and urocanic acid [UCA]).[11] These components, known collectively as the natural moisturizing factor (NMF), are important in regulating SC homeostasis. They act as "nature's humectant" to retain physiologic epidermal water content and maintain the structural integrity of keratin bundles in corneocytes essential for reducing TEWL.[12] Importantly, other hydrolytic enzymes in the SC are responsible for desmolytic activity, which allows for

the invisible process of normal skin desquamation via individual separation of corneocytes. When there is diminished SC water content below a physiologic level, these hydrolytic enzymes function sub-optimally, leading to incomplete separation and clumping of individual corneocytes, which can be perceived visibly as scaling.[2]

Topical skincare products, including creams, lotions, and ointments have been widely used to protect and maintain skin barrier structure and function. A major emphasis of optimized moisturizer formulations has been to incorporate ingredients that reduce TEWL (occlusives, NMFs) and to replenish and/or support functional components (NMFs, ceramides antioxidants, others).[4,13] Due to their established major importance in skin barrier functions, and their impairment and/or quantitative decrease in several environmental or disease-associated scenarios, ceramides are frequently included ingredients in many topical skin moisturizers; many formulations also contain NMFs to further enhance the maintenance of skin barrier function by including compounds found in the innate epidermal barrier.[4,13,14]

In the present study, we describe the head-to-head clinical comparison of 2 ceramide plus NMF-enriched formulations (cream and lotion) and a ceramide-based formulation (cream) on skin hydration in 2 double-blinded comparative studies after 10 days of daily bilateral application followed by 5 days of no moisturizer use for regression analysis.

MATERIALS AND METHODS

Test Products

Study 1: Moisturizer A, Ceramide plus natural moisturizing factor-enriched cream (Eucerin Advanced Repair Cream, Beiersdorf Inc.); Moisturizer B, Ceramide-based cream (CeraVe Moisturizing Cream, L'Oreal Group).

Study 2: Moisturizer C, Ceramide plus natural moisturizing factor-enriched lotion (Eucerin Advanced Repair Lotion, Beiersdorf Inc.); Moisturizer B, Ceramide-based cream (CeraVe Moisturizing Cream, L'Oreal Group).

Institutional Review Board

Prior to participant enrollment for the study, the protocol and informed consent form (ICF) for this study were reviewed and approved by IntegReview Institutional Review Board (IRB) on September 26, 2016. IntegReview IRB, located in Austin, Texas, is a duly constituted IRB under Title 21 Code of Federal Regulations (CFR) Parts 50 and 56.

Informed Consent

Written informed consent conforming to 21 Code of Federal Regulations (CFR) 50.25 was obtained from each adult participant (at least 18 years old) or the parent/guardian of each minor participant (under the age of 18 years) prior to enrollment in the study. The original signed ICF for each patient participating in the study was retained in the study file and each patient received a copy of the signed form.

Study Design

Both studies were bilateral and double-blinded and were conducted from October 5, 2016, to November 2, 2016, at the Thomas J. Stephens & Associates, Inc. Colorado Research Center, Colorado Springs, Colorado.

Participants

Study 1: The first study included 35 participants (Male, n=6; Female, n=29) ages 16-70 years old (Mean, 57.1); 25% of the group (n=9) had mild dryness on the legs, and approximately 75% (n=26) had moderate-to-severe dryness on the legs.

Study 2: The second study included 33 participants (Male, n=12; Female, n=21) ages 16-70 years old (Mean, 50.9). Approximately 50% of the group (n=16) had mild dryness, and approximately 50% (n=17) had moderate-to-severe dryness on the legs.

Test Area and Application Procedures

Study 1: During the 10-day usage period of the study, participants applied Moisturizer A on the randomly assigned (right or left) leg and Moisturizer B on the opposite leg, once daily as directed. After 10 days, participants discontinued the use of the moisturizer and participated in a 5-day regression period during which time no moisturizer was applied.

Study 2: During the 10-day usage period of the study, participants applied Moisturizer B on the assigned (right or left) leg and Moisturizer C on the opposite leg, once daily as directed. After 10 days, participants discontinued the use of the moisturizer and participated in a 5-day regression period during which time no moisturizer was applied.

Enrollment Procedures

Prior to the start of the study, potential participants were screened over the telephone for eligibility criteria. Female and male participants between ages 16 and 70 years old were scheduled for eligibility screening at the clinic. Prospective participants were advised not to shave their legs and to avoid the application of any topical moisturizing product and use of cleansers with moisturizing properties on the legs for at least 3 days prior to visit 1.

At visit 1 (baseline), each prospective participant or the parent/guardian of minor prospective participants read and signed the informed consent form after the nature of the study was explained and any study-related questions were answered. Minor prospective participants (under the age of 18 years) signed an assent form indicating their agreement to participate

in the study. In addition, prospective participants or parents/guardians completed an eligibility and health questionnaire. Prospective participants who signed the initial paperwork were assigned a screening number.

Prospective participants acclimated to ambient temperature and humidity conditions for at least 15 minutes prior to participating in clinical procedures. During the study, applicable waiting/instrumentation rooms were maintained at a temperature of 68°F to 75°F and relative humidity from 35% to 65%.

Application of Moisturizers

Participants were distributed pre-weighed units of the test material and instructed to apply it to each leg according to the following instructions:

- Apply a sufficient amount of moisturizer (about the size of a half-dollar coin) to the appropriate leg (starting at the kneecap and down to the ankle) once daily in the evening for 10 days. Please make sure the moisturizer covers the entire lower leg area. Wash and dry hands between applications of each test material (one moisturizer per leg) to avoid cross-contamination of the sites.

Regression Period

After completion of the day 10 procedure, participants were instructed not to shave their legs, apply any moisturizing products, or use cleansers with moisturizing effects on the legs for a 5-day regression period during which no moisturizer was used.

Corneometer Measurements

At baseline, day 10, and day 15, triplicate corneometer (CM 825, Courage & Khazaka, Germany) measurements were taken on both legs of each participant at the midline between the knee and ankle of the lateral side.

Statistical Analysis

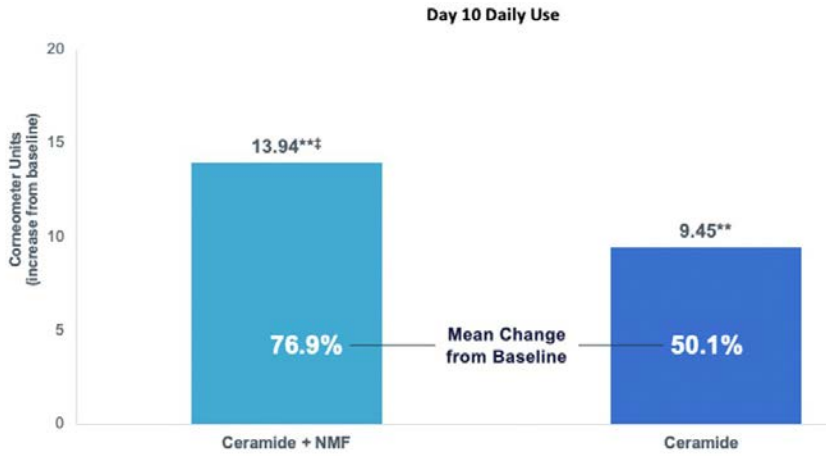
Triplicate corneometer measurements for each participant, location, and time point were averaged prior to statistical analysis. All results are presented as the percent mean change from baseline. Significance of mean change from baseline and comparison between groups was tested using a paired t-test. All statistical tests were 2-sided at a significant level of alpha=0.05. *P*-values were reported to 3 decimal places. No multiple testing corrections were considered in the study. Statistical analyses were performed using SAS software version 9.40 series (SAS Statistical Institute).

RESULTS

Ceramide Plus NMF Cream vs Ceramide Cream

Assessment of skin hydration by corneometer was performed on day 10 after once daily use of ceramide plus NMF or ceramide cream, and a 5-day regression period of no daily moisturization (day 15). Daily moisturization for 10 days with the ceramide plus NMF cream resulted in a 76.9% increase in corneometer units compared to baseline ($P<0.001$), while daily moisturization of the ceramide cream resulted in an increase of 50.1% compared to baseline ($P<0.001$), with an overall statistical significance in favor of the ceramide plus NMF cream vs the ceramide cream ($P<0.001$) (Figure 1).

FIGURE 1. Clinical evaluation of skin hydration after 10 days of daily moisturization. Thirty-five participants (Male, n=6; Female, n=29) ages 16 to 70 years old (Mean, 57.1), 25% (n=9) with mild dryness on the legs and approximately 75% (n=26) with moderate-to-severe dryness on legs, applied ceramide plus NMF cream on the assigned (right or left) leg and ceramide cream on the opposite leg, once daily for 10 days, as directed. At baseline and day 10, triplicate corneometer (CM 825, Courage & Khazaka) measurements on both legs of each participant were taken at the midline between the knee and ankle of the lateral side and increase from baseline corneometer units were plotted, with mean change from baseline calculated.



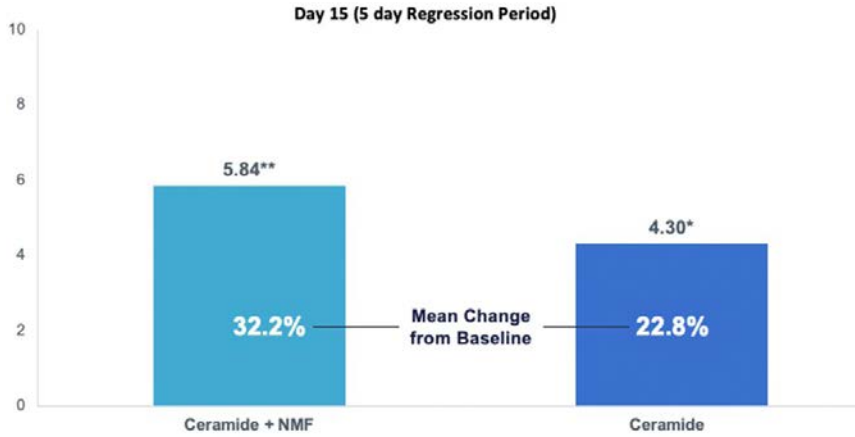
**Statistically significant improvement compared to baseline ($P<0.001$). ‡Statistically significant difference between treatments in favor of Ceramide plus NMF ($P<0.001$).

The lasting effect of Improvement in skin hydration was evaluated at day 15 (5-day regression with no additional moisturization). The ceramide plus NMF cream 10-day moisturization plus 5-day regression yielded a 32.2% increase from baseline corneometer units (P<0.001), while the ceramide cream yielded a 22.8% increase from baseline corneometer units (P<0.05) (Figure 2).

Ceramide Plus NMF Lotion vs Ceramide Cream

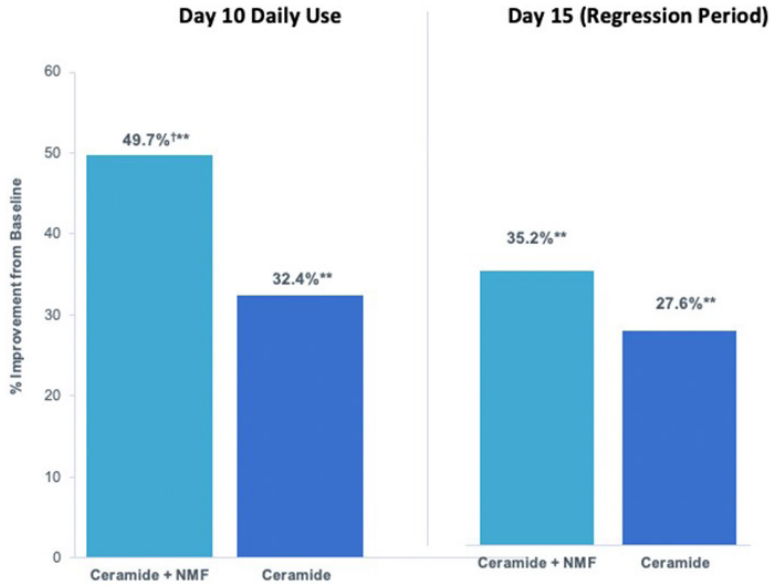
To address the misconception that creams are always more efficacious than lotions, we evaluated the clinical efficacy of improvement in skin hydration after 10 days of daily use of the ceramide plus NMF lotion compared to the ceramide cream, followed by corneometer assessment at day 15 following a 5-day

FIGURE 2. Clinical evaluation of skin hydration on day 15 (5-day regression period with no moisturization). Thirty-five participants (Male, n=6; Female, n=29) ages 16 to 70 years old (Mean, 57.1), 25% with (n=9) mild dryness on the legs and approximately 75% (n=26) with moderate-to-severe dryness on the legs, applied ceramide plus NMF cream on the assigned (right or left) leg and ceramide cream on the opposite leg, once daily for 10 days, as directed, followed by a 5-day regression period of no moisturization. At baseline and day 15, triplicate corneometer (CM 825, Courage & Khazaka) measurements were taken on both legs of each participant at the midline between knee and ankle of the lateral side, and increase from baseline corneometer units were plotted, with mean change from baseline calculated.



**Statistically significant improvement compared to baseline (P<0.001). *Statistically significant improvement compared to baseline (P<0.05).

FIGURE 3. Clinical evaluation of skin hydration after 10 days of daily moisturization and on day 15 (5-day regression period with no moisturization). Thirty-three participants (Male, n=12; Female, n=21) ages 16 to 70 years old (Mean, 50.9), some with mild dryness on the legs (n=16) and some with moderate-to-severe dryness on legs (n=17), applied ceramide plus NMF lotion on the assigned (right or left) leg and ceramide cream on the opposite leg, once-daily as directed for 10 days followed by a 5-day regression period of no moisturization. At baseline, day 10, and day 15, triplicate corneometer (CM 825, Courage & Khazaka) measurements were taken on both legs of each participant at the midline between the knee and ankle of the lateral side, and increase from baseline corneometer units were plotted, with mean change from baseline calculated.



**Statistically significant improvement compared to baseline (P<0.001). †Statistically significant difference between treatments in favor of Ceramide + NMF lotion (P<0.05).

regression period, similar to the methodologies used in the ceramide plus NMF cream vs ceramide cream study described above. Daily moisturization for 10 days with the ceramide plus NMF lotion resulted in a 49.7% increase in corneometer units compared to baseline (P<0.001), while daily moisturization of the ceramide cream resulted in an increase of 32.4% compared to baseline (P<0.001), with an overall statistical significance in favor of the ceramide plus NMF lotion vs the ceramide cream (P<0.05) (Figure 3). Once again, the lasting effect of improvement in skin hydration was evaluated at day 15 (5-day regression with no additional moisturization). The ceramide plus NMF lotion 10-day moisturization plus 5-day regression yielded a 35.2% increase from baseline corneometer units (P<0.001), while the ceramide cream yielded a 27.6% increase from baseline corneometer units (P<0.05) (Figure 3).

DISCUSSION

While the focus on barrier repair with topical moisturization has often centered on the inclusion of ceramides in formulations, the utilization of additional ingredients, including NMFs, has not received adequate attention to their potential importance in moisturizer formulations. In this study, we demonstrate the statistical superiority of both a ceramide plus NMF cream and a ceramide plus NMF lotion compared to a ceramide cream based on recognized objective methods of testing hydration after 10 days of daily application. While all 3 formulations improved skin hydration, as indicated by the significant increase in corneometer units from baseline, the ceramide plus NMF cream and ceramide plus NMF lotion demonstrated a significantly higher increase in skin hydration as compared to baseline and as compared to the ceramide cream.

Ceramides and NMFs are major SC components that contribute to maintaining skin hydration.[15] NMFs originate from the breakdown of filaggrin and include hydrophilic amino acids, and their derivatives such as PCA, UCA, lactic acid, sugars, organic acids, peptides, and urea, which collectively are associated with the establishment of a physiologic water gradient in the SC.[16-18]

The increased role of NMFs in skin barrier function continues to be elucidated, with recent studies demonstrating the importance of NMFs in skin barrier function in atopic dermatitis (AD).[19] Decreases in NMF are attributed to both intrinsic and extrinsic factors, which are associated with xerotic skin, increased surface pH, and increased risk for worsening of AD.[19] The addition of NMFs in well-formulated topical moisturizers has previously been shown to augment skin hydration by supporting the maintenance of water content in dehydrated conditions, thus retaining the optimal fluidity and function in SC lipid and protein components.[20] Exposure of the skin to commonly encountered external daily conditions that can impair epidermal barrier function, including washing, showering, and bathing, or the use of occlusive sanitary products such as diapers, which can deplete important hydrophilic compounds of the SC, including NMFs. This depletion markedly affects the ability of the SC to attract and maintain water, leading to a cascade of events that can steadily progress to severe xerosis and eczematous dermatitis, both associated with pruritus and increased potential for microbial colonization.[21,22] These data and the results of our study suggest that the utilization of a moisturizer (cream or lotion) that contains both ceramides and NMFs may be a more optimal formulation, compared to a ceramide cream alone, for maintaining skin hydration, improving barrier function, and decreasing the clinical sequela associated with dry skin.

DISCLOSURES

Dr Baldwin has acted as an investigator, consultant, and/or speaker for Almirall, Bausch, Cassiopea, EPI Health, Galderma, La Roche-Posay, L'Oreal, Mayne Pharma, Sol-Gel, Sun Pharma, and Vyne. Dr Del Rosso has served as a consultant, investigator, and/or speaker for Abbvie, Almirall, Amgen, Arcutis, Bausch Health (Ortho Dermatologics), Beiersdorf, Dermavant, EPI Heath, Ferndale, Galderma, Incyte, JEM Health, LaRoche Posay, LEO Pharma, Lilly, Loreal, MC2 Therapeutics, Novan, Pfizer, Regeneron, Sanofi, Se0nte, SolGel, Sun Pharma, UCB and Unilever. Craig Arrowitz is an employee of Beiersdorf Inc.

REFERENCES

1. Moroki T. Morphological characteristics and notes of the skin in preclinical toxicity assessment. *J Toxicol Pathol.* 2023;36:85-94.
2. Del Rosso JQ, Levin J. The clinical relevance of maintaining the functional integrity of the stratum corneum in both healthy and disease-affected skin. *J Clin Aesthet Dermatol.* 2011;4:22-42.
3. Green M, Kashetsky N, Feschuk A, et al. Transepidermal water loss (TEWL): Environment and pollution-a systematic review. *Skin Health Dis.* 2022;2:e104.
4. Purnamawati S, Indrastuti N, Danarti R, et al. The role of moisturizers in addressing various kinds of dermatitis: A review. *Clin Med Res.* 2017;15:75-87.
5. Akdeniz M, Gabriel S, Lichterfeld-Kottner A, et al. Transepidermal water loss in healthy adults: A systematic review and meta-analysis update. *Br J Dermatol.* 2018;179:1049-1055.
6. Ananthapadmanabhan KP, Leyden JJ, Hawkins SS. Recent advances in mild and moisturizing cleansers. *J Drugs Dermatol.* 2019;18:s80-s88.
7. Sparr E, Bjorklund S, Dat Pham Q, et al. The stratum corneum barrier-from molecular scale to macroscopic properties. Current Opinion in Colloid & Interface *Science.* 2023;67:101725.
8. Pappas A. Epidermal surface lipids. *Dermatoendocrinol.* 2009;1:72-76.
9. Proksch E, Brandner JM, Jensen JM. The skin: An indispensable barrier. *Exp Dermatol.* 2008;17:1063-1072.
10. Harding CR. The stratum corneum: Structure and function in health and disease. *Dermatol Ther.* 2004;17 Suppl 1:6-15.
11. Rawlings AV, Scott IR, Harding CR, et al. Stratum corneum moisturization at the molecular level. *J Invest Dermatol.* 1994;103:731-741.
12. Szakall A. Properties, origin, and physiological functions of the substances of the keratinized epidermis determining the hydrogen-ion concentration. *Arch Klin Exp Dermatol.* 1955;201:331-360.
13. Weber TM, Kausch M, Rippke F, et al. Treatment of xerosis with a topical formulation containing glyceryl glucoside, natural moisturizing factors, and ceramide. *J Clin Aesthet Dermatol.* 2012;5:29-39.
14. Draelos ZD. Modern moisturizer myths, misconceptions, and truths. *Cutis.* 2013;91:308-314.
15. Jung K, Kim SH, Joo KM, et al. Oral intake of enzymatically decomposed AP collagen peptides improves skin moisture and ceramide and natural moisturizing factor contents in the stratum corneum. *Nutrients.* 2021;13(12):4372.
16. Bouwstra JA, Dubbelaar FE, Gooris GS, et al. The role of ceramide composition in the lipid organisation of the skin barrier. *Biochim Biophys Acta.* 1999;1419:127-136.
17. Scott IR , Harding CR. Filaggrin breakdown to water binding compounds during development of the rat stratum corneum is controlled by the water activity of the environment. *Dev Biol.* 1986;115:84-92.
18. Scott IR, Harding CR, Barrett JG. Histidine-rich protein of the keratohyalin granules. Source of the free amino acids, urocanic acid and pyrrolidone carboxylic acid in the stratum corneum. *Biochim Biophys Acta.* 1982;719:110-117.
19. Basu MN, Mortz CG, Jensen TK, et al. Natural moisturizing factors in children with and without eczema: Associations with lifestyle and genetic factors. *J Eur Acad Dermatol Venereol.* 2022;36:255-262.
20. Gunnarsson M, Mojumdar EH, Topgaard D, et al. Extraction of natural moisturizing factor from the stratum corneum and its implication on skin molecular mobility. J Colloid Interface Sci. 2021;604:480-491.
21. Bjorklund S, Pham QD, Jensen LB, et al. The effects of polar excipients transcutol and dexpanthenol on molecular mobility, permeability, and electrical impedance of the skin barrier. *J Colloid Interface Sci.* 2016;479:207-220.
22. Pham QD, Bjorklund S, Engblom J, et al. Chemical penetration enhancers in stratum corneum - relation between molecular effects and barrier function. *J Control Release.* 2016;232:175-187.

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