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Effects of Emulsion's Temperature Characteristics on the Changes in Skin Conditions

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Abstract

To find a method to increase the percutaneous absorption rate, this study observed the changes in skin conditions by changing the temperature characteristics of an emulsion. This study conducted an in vitro percutaneous absorption experiment and a clinical trial using two types of emulsions with different temperature characteristics. The changes in skin temperature, blood circulation, oil, and moisture were evaluated, and the results of the clinical trial were compared with the results of the in vitro experiment. The results of the in vitro percutaneous absorption test showed that the permeability of high-temperature emulsion increased by 21.5%. Moreover, the results of the clinical trial revealed that blood circulation, moisture, and oil content of the treatment group increased by 38.34%, 76.99%, and 122.68%, respectively, and they were significantly different between the two groups. The result showed that the emulsion's temperature characteristics increased the percutaneous absorption rate and could improve skin conditions. The results will be helpful for developing the characteristics of functional cosmetics.

Keywords: Emulsion's temperature; Skin barrier; Blood circulation; Moisture; Percutaneous absorption rate

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Introduction

Many physiological studies on skin, hair, and nails are needed for developing cosmetic formulations. The skin is a complex multi-layered organ that makes up 7%–10% of the total body weight and is composed of five different cell types, immunocytes, and nerve cells. Moreover, it covers the entire body, and each body part has a different skin thickness (Miller LK *et al.*,2011). The main function of the skin is to provide a solid structural barrier to protect the

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internal tissues from the external environment and not to act as a conduit for external chemicals to penetrate into the body. The barrier function is the most distinctive characteristic of the stratum corneum. The skin maintains homeostasis by preventing moisture loss from the skin, outflow of other intrinsic endogenous substances, and inflow of foreign substances from the outside (Mah CS *et al.,* 2012). The skin also acts as a chemical barrier owing to the highly organized multi-layered overlapping cells sealed by dense intercellular lipid multilamellar as well as a structural barrier (Tóth KF*et al.,* 2019).Furthermore, it has a complex structure with dendritic cells and macrophages. Consequently, the skin hinders the effective application of most cosmetics. Furthermore, due to the same reason, only a limited number of cosmetic ingredients can be percutaneously delivered (Tóth B.*et al.,* 2014; Tri s., 2019). Numerous studies have been conducted for overcoming the barrier characteristics for the penetration of cosmetic ingredients. Various factors affect the percutaneous absorption. The size of a molecule is the most important factor. In general, molecules less than 500 Da can passively diffuse through the skin. When a molecule is equal to or larger than 500 Da, passive permeability rapidly decreases. Therefore, various methods such as chemical reinforcing agents, iontophoresis, formulation vehicles, and microneedles may be used. In addition, skin age and the distribution of adnexa are biological factors that greatly influence the penetration of cosmetic ingredients (Heather A.*et al.,* 2005; MarzoVDi, 2018; Trisha K., 2019).

Along with these studies, accurately analyzing skin penetration is important. As a method of evaluating the skin permeability amount of an active substance, the relative absorption of a compound can be examined using an important parameter, “flex”. The diffusion of an active cosmetic ingredient is the main movement mechanism for crossing the membrane for percutaneous absorption(Karadzovska D.*et al.,* 2013). Therefore, it can be explained by Fick’s first diffusion law. In addition, the diffusion coefficient is determined by numerous variables, such as temperature, the viscosity of a solution, and the size of the solute, to influence the diffusion velocity(Friend DR*et al.*, 1992).

Interest and demand for various functional cosmetics are increasing in recent years. However, many functional cosmetics do not show expected efficacy because they are not often absorbed well into the skin, although their functional ingredients have excellent efficacy. Therefore, this study aims to support the development of cosmetic products and devices that increase the percutaneous absorption rate by comparing and analyzing the changes in percutaneous absorption rates and skin conditions according to temperature characteristics, one of the factors that determine the diffusion coefficient.

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Materials and Methods

Preparation of emulsion formulation

To prepare samples, oil and water phases, which were composed of cholesterol, butylene glycol, and ethanol, were dissolved by heating to 70–75 ℃. Afterward, the oil phase was added to the water phase while emulsifying it at 3,000 rpm for 10 min using a homogenizer to prepare an emulsion. Niacinamide was used as an active substance.

Percutaneous absorption test

An in vitro percutaneous absorption experiment (Franz diffusion cell method) was conducted using the Franz diffusion cell system (FDC-6T, Logan Instrument, USA) to evaluate the percutaneous absorption rate of niacinamide emulsion. This experiment used artificial skin (Strat-M membrane, Merck Millipore, USA), and the membrane was fixed between the donor and receptor phases with the stratum corneum facing upward. The receptor chamber was filled with 50% ethanol, and the temperature was maintained at 37℃ and 41℃ using a water bath. The niacinamide content in the sample collected from the receptor chamber after 24 h was quantified using HPLC. In addition, to measure the niacinamide content remaining on the membrane after 24 h, the membrane was washed three times with PBS. After washing, the part that did not contact the receptor phase was cut out, and the remaining part was shredded using scissors. The shredded membrane was placed to 10 mL of 50% ethanol and extracted and treated using an ultrasonic cleaner for 1 h to quantify the niacinamide content remaining on the membrane.

Human clinical trial

Ten women in their 30s–40s were the study participants; they voluntarily filled out the consent form for the clinical study after understanding the objectives and contents of the study. The experiment was conducted with the approval of the institutional review board. The prepared sample emulsion was applied on the treatment and control groups. Afterward, the participants of the control group were covered with a nonwoven fabric as a sheet mask to maintain room temperature, whereas the participants of the treatment group were kept at 37°C using a heat pack. After one application for 10 min, changes in skin temperature, blood circulation, oil, and moisture were compared and evaluated. Skin temperature, thermal imaging, blood flow, moisture, and oil were measured using FLIR T620® (FLIR systems AB, Sweden), FLIR R&D software, moor FLPI-2TM (moor instruments, UK), Corneometer® CM825 (C+K, Germany), and Sebumeter® SM810 (C+K, Germany), respectively.

Data processing

SPSS Ver.26 (IBM, USA) was used to analyze the data, and Shapiro–Wilk test was used to test for normality of the data. The homoscedasticity between the groups for all evaluation items was tested by independent t-test ($p > 0.1$). Paired t-test was

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used to compare the results before and after product use, and independent t-test was used to compare the groups ($p > 0.05$).

Results and Discussion

Results of percutaneous absorption test

The in vitro skin absorption test method is a way to measure the quantity of a test substance passed through the skin to the solution reservoir. Furthermore, it can use human skin or the skin of another species, repeatedly measure the test substance, and evaluate exposure conditions without using laboratory animals. Moreover, the advantages of this method are that a wide range of test substances can be used for the test and that the relationship between skin damage and skin absorption, which cannot be evaluated by in vivo tests due to ethical reasons, can be evaluated (HarunaT.*et al.*, 2017) Fig. 1 shows the test results of the application of the two emulsion temperatures. It was confirmed that the temperature of the emulsion was 32℃ and 36℃ when the temperature of the water bath was maintained at 37℃ and 41℃, respectively. When the experiment was conducted for 10 min, the difference in transmittance between them was 36.9% after 10 min and 21.5% after 15 min, indicating that the permeability rate increased at high temperatures. The high-temperature high-frequency device is used to increase the percutaneous absorption rate based on this principle and many methods have been used to increase it. In other words, proper heat can maximize the penetration of active substances by opening pores(DilekB.*et al.*,2013).

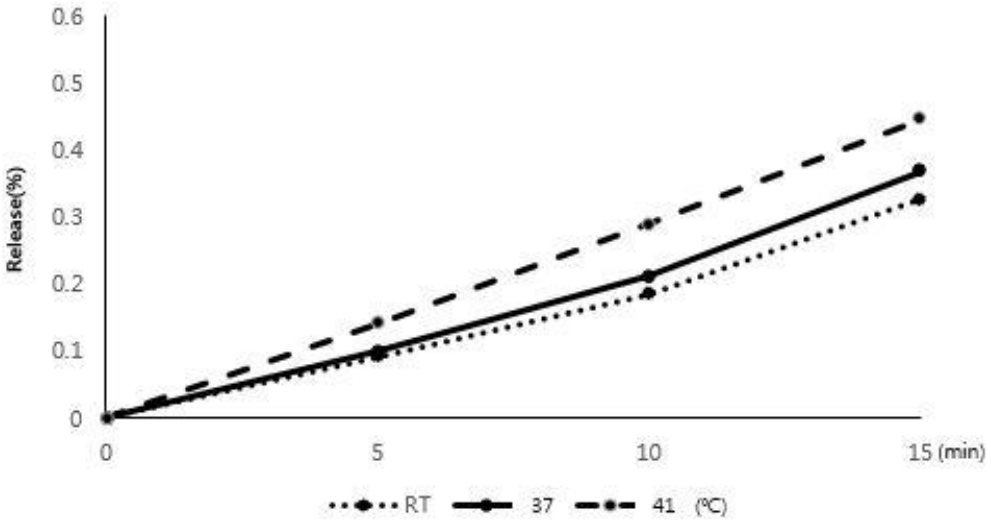

Figure 1. Result of permeation absorption test (15min)

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Results of clinical trial

Safety evaluation

Safety was checked by observing the test site of study subjects after applying the emulsion prepared prior to this experiment and conducting a questionnaire. The attachment part did not show any adverse reactions, and no skin trouble was observed during the test period. This study evaluated the homogeneity of the treatment and control group, and the results showed that skin temperature, blood circulation, oil, and moisture were not significantly different between the two groups before the application of the product ($p > 0.1$). Therefore, we judged that their before-test conditions were identical.

Skin temperature change

Figure 2 shows the changes in skin temperature before application, 5 min after application, 10 min after application, and after removal of the sample product for each group. Both cheek areas were measured three times and the mean value of the measurements was used to analyze skin temperature changes. When comparing with the before emulsion application, the skin temperature of the treatment group significantly ($p < 0.05$) increased by 1.45%, 1.45%, and 3.63% after 5 and 10 min of application and after removal, respectively. In the control group, it significantly ($p < 0.05$) decreased by 18.33%, 15.87%, and 12.97% after 5 and 10 minutes of application and after removal, respectively. The results revealed that the treatment group (with heat application) showed a temperature increase, while the control group (without heat application) showed temperature decrease due to moisture evaporation.

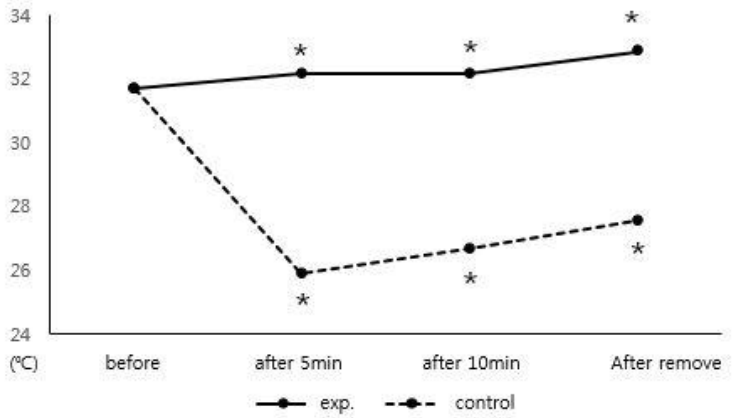

Figure 2. Result of skin temperature change (*p< 0.05)

Changes in skin blood circulation

Table 1 shows the changes in skin blood circulation before and after the application of the

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sample product for each group, and Table 1 shows the changes between the groups. Twenty blood circulation images were taken on the face at 3-s intervals for 1 min, and they were analyzed by averaging the values of both cheeks. The blood circulation of the treatment group significantly increased by 38.34% ($p < 0.05$), whereas that of the control decreased. Thus, the groups were significantly different ($p < 0.05$).

When the blood flow of the skin increases, the functions of cells are enhanced and the defense mechanism of the body is improved. Consequently, metabolism is promoted. Moreover, as the blood flow in the capillaries increases, the supply of oxygen, nutrients, antibodies, and leukocytes increases and lymphokinesis is promoted to increase the discharge of waste. In addition to these physiological effects, an environment suitable for the penetration of the cosmetics' active substances is created, as described above, to increase the permeability of active substances (Muluye RA*et al.*,2014;Dyson M., 1982). Consequently, it can further improve the skin. In other words, the permeability of active substances can be changed due to the difference in blood circulation between the two groups.

Table 1: Changes in skin blood circulation before and after the application of the sample product

group	Time point	mean	SD	*p*-value	%
Exp.	before	100.43	24.37	0.002*	38.34▲
	after	138.93	32.39		
Cont.	before	113.13	22.71	0.021*	15.74▽
	after	95.32	2085		

Changes in skin oil and moisture

Figures 3 and Table 2 show the changes in skin oil and moisture content before and after the removal of the sample product for each group. The moisture content of the treatment group significantly increased by 76.99% ($p < 0.05$) and its sebum content of significantly increased by 122.68% ($p < 0.05$). The moisture and oil contents of the control group increased by 31.03% and 17.92%, respectively, indicating significant differences between the two groups. We believe that the difference is due to the increase in the permeability of active substances owing to the thermal effect of the treatment group. The results suggest that the temperature of the emulsion affected the diffusion coefficient.

As explained by Fick's diffusion law (equation 1), the diffusion of an active substance can be explained by the differential concentration gradient over the path length for a given time. Therefore, it can be simplified as Equation (2). Where, c is the concentration of the emulsion, h

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is the diffusion path, and k is the diffusion coefficient between the emulsion and skin. However, the introduction of a diffusion coefficient term is inevitable as the accurate estimation of D and h in a specific membrane, such as the skin, is impossible (Tobin DJ., 2006; KupczewskaDM*et al.*, 2010). Therefore, in this case, biological factors affecting diffusion are very important, and particularly, the flow of blood circulation can be an important factor. In other words, the temperature change of the emulsion can be an important factor in the penetration of an active substance. However, since an excessive high-temperature can cause skin aging and inflammation, maintaining an appropriate temperature and time is critical (Weiberger A.*et al.*, 1989).

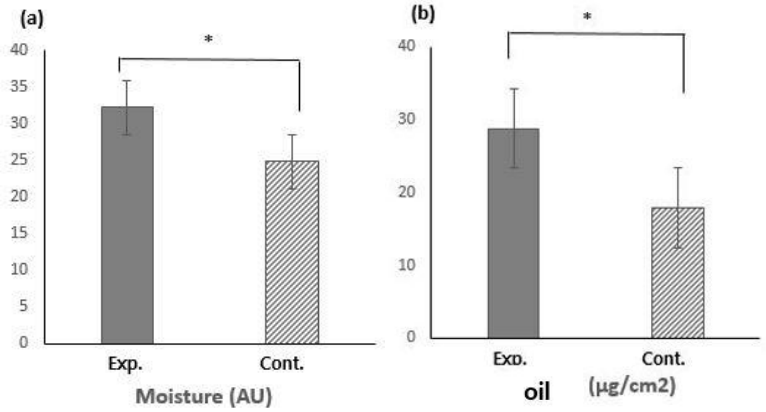
$$J = -D\frac{KC}{h} \qquad (1)$$

$$P = D\frac{K}{h} \qquad (2)$$

Table 2: Changes in skin circulation(moisture, oil) before and after the application of the sample product

	group	mean	SD	*p*-value
moisture	Exp.	37.20	4.82	0.006*
	Cont.	24.80	13.99	
oil	Exp.	28.83	18.56	0.029*
	Cont.	17.92	15.81	

* $p < 0.05$



* $p < 0.05$

Figure 3. Changes in skin condition(moisture, oil)
(a: moisture(AU), b : oil(μg/cm^2))

Conclusion

This study aimed to evaluate the effects of emulsion's temperature characteristics on the changes in percutaneous absorption and skin conditions. The results of an in vitro experiment confirmed that permeability increases at high temperatures. The results of the clinical trial also showed an increase in skin blood circulation, oil, and moisture when applying a high-temperature emulsion. These results showed that an increase in the emulsion's temperature positively affected the percutaneous permeability, thereby improving the skin condition. As the demand for cosmetics continues to grow and people have increasing needs, products with better efficacy are desired. More studies are needed to develop high-functional substances and methods for increasing the permeability of cosmetic products. Furthermore, their safety must be guaranteed.

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References

1. Dilek B, Gözm B, Ahin E, Baydar M, Ergör G.E.O, Bircan C, Gülbahar S., 2013. Efficacy of paraffin bath therapy in hand osteoarthritis a singleblinded randomized controlled trial. *Arch Phys Med Rehabil*, 94(4), 642-649.DOI:10.1016/j.apmr.2012.11.024
2. Dyson M., 1982. Non-thermal cellular effects of ultrasound. *British Journal of Cancer*, 45(1), 165-171.
3. Friend D.R., 1992. In vitro skin permeation techniques. *J Control Release*, 18(3), 235–248.DOI:10.1016/0168-3659(92)90169-R
4. Heather A, Benson E., 2005. Transdermal drug delivery penetration enhancement techniques. *Current Drug Delivery*, 2(1), 23-33.DOI:10.2174/1567201052772915
5. Haruna T, Soga M, Morioka Y, Imura K, Furue Y, Yamamoto M, Hayakawa J, Deguchi M, Arimura A, Yasui K., 2017. The Inhibitory Effect of S-777469, a Cannabinoid Type 2 Receptor Agonist, on Skin Inflammation in Mice. *Pharmacology*, 99(5-6), 259-267.DOI:10.1159/000455916
6. Karadzovska D, Brooks J.D., Monteiro-Riviere N.A., Riviere J.E., 2013. Predicting skin permeability from complex vehicles. *Adv Drug Deliv Rev*, 65(2), 265–277.DOI:10.1016/j.addr.2012.01.019
7. Kupczewska-Dobecka M, Jakubowski M, Czerczak S., 2010. Calculating the dermal flux of

chemicals with OELs based on their molecular structure: An attempt to assign the skin notation. *Environ ToxicolPharmacol*, 30(2), 95–102.DOI:10.1016/j.etap.2010.06.005

8. Mah C.S., Kochhar J.S., Ong P.S., Kang L.,2012. A miniaturized flow-through cell to evaluate skin permeation of endoxifen. *Int J Pharm*, 441(1), 433–473.DOI:10.1016/j.ijpharm.2012.11.011
9. Miller L K, Devi L A. 2011. The highs and lows of cannabinoid receptor expression in disease: mechanisms and their therapeutic implications. *Pharmacol Rev*, 63(3), 461-70.DOI:10.1124/pr.110.003491
10. Muluye R A, Bian Y, Alemu P N. 2014. Anti-inflammatory and Antimicrobial Effects of Heat-Clearing Chinese Herbs: A Current Review. *J Tradit Complement Med*., 4(2), 93-98.DOI:10.4103/2225-4110.126635
11. Tóth B, Attila O A, Zöllősi A G, Tamás B. 2014. TRP Channels in the skin. *Br J Pharmacol*. 171(10), 2568–2581.DOI:10.1111/bph.12569
12. Tobin D J. 2006. Biochemistry of human skine our brain on the outside. *ChemSoc Rev.*, 35(1), 52-67.DOI:10.1039/B505793K
13. Trisha K. 2019. A study on knowledge and attitude towards digital health of rural population of india- Innovations in practice to improve healthcare in the rural population. Int. *J of EmergingMultidisciplinary Research*, 3(3), 13-21.
14. Tóth K F, Ádám D, Bíró T, Olah A. 2019. Cannabinoid Signaling in the Skin: Therapeutic Potential of the "C(ut)annabinoid" System. *Molecules*, 24(5), 918-925.DOI:10.3390/molecules24050918
15. Tri S, Asep B D N. 2019. Economic Evaluation on Production of ZnO Nanoparticles from Laboratory Scale to Industrial Scale. Int. *J of Emerging Multidisciplinary Research*,3(4), 19-24.
16. Marzo V Di. 2018. New approaches and challenges to targeting the endocannabinoid system. *Nat Rev Drug Discov.*, 17(9), 623-39.DOI:10.1038/nrd.2018.115
17. Weiberger A, Falilah R, Pinkhas J. 1989. Intra-articular temperature measurements after superficial heating. *Scand J Rehabil Med*., 21(1), 55-57.