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피부장벽에 대한 Bio-Mimic Liquid Crystal Emulsion (BLCE)의 긍정적 효과에 관한 연구

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Study on the beneficial effect of Bio-Mimic Liquid Crystal Emulsion (BLCE) on Skin Barrier Function

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요 약: 최근 기능성화장품과 피부관련 의약품 분야에서 multi-lamellar와 liquid crystal의 구조가 커다란 관심을 끌고 있다. 결합수와 고정된 유상을 함유하는 multi-lamellar 구조는 피부의 세포간지질의 lamellar 구조의 재건과 보습을 유지하는 작용으로써 보습기능이나 장벽기능의 복구에 우수하며, 이 지질들은 각질층에 침투하여 유지된다. 본 연구에서 고급 지방 알코올, 레시틴, 콜레스테롤을 사용하여 제조한 bio-mimic liquid crystal emulsion (BLCE)의 피부장벽 기능을 측정하였고 일반적인 계면활성제와 세포독성을 비교하였다.

Abstract: The multi-lamellar and liquid crystal structures have drawn great public attention in the functional cosmetic and skin-related medicinal areas recently. The structure of an emulsion containing aqueous phase as a binding water and fixed oil phase components forming an association compound of the multi-lamellar structure can reconstruct the intercellular lipid lamellar structure in the stratum corneum and restore barrier function of the skin. In this study, we investigated the beneficial effect of bio-mimic liquid crystal emulsions (BLCE) containing higher fatty alcohol, lecithin, and cholesterol on the skin barrier function, and evaluated its cytotoxicity.

Keywords: *liquid-crystal, multi-lamellar, skin barrier, trans-epidermal water loss (TEWL), BLCE*

1. Introduction

Generally, materials have regular particle arrangements in solid state, and become irregular when they transform into liquid state. Some materials are regular in its molecular arrangement but are liquid at the same fluid. They are called liquid crystals. Those liquid crystalline phases (mesomorphic) are categorized into thermotropic and lyotropic. Thermotropics are classified into smectic, nematic, and cholesteric according to the arrangement of the bar-type molecules of liquid crystal, and lyotropics are classified into cubic (isotrpoic), lamellar (neat) and hexagonal according to the shapes of molecules. The structure of an emulsion containing aqueous phase as a binding water and fixed oil phase components forming an association compound of the multi-lamellar structure can reconstruct the intercellular lipid lamellar structure in the stratum corneum. Restoration of the moisturization function or barrier function of the skin as the function for maintaining moisture is superior. The lipid is penetrated into and maintained in the stratum corneum.

Concerning the crystal shape of higher fatty alcohol, liquid crystal is shaped in a hexagonal state, and crystals state, which have pearl effects, are shaped in a monoclinic state. In the case of emulsion products,

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the highest viscosity is shown a few hours after manufacture. This is because of the regular arrangement of the formerly irregular liquid crystal phase because of the newly formed liquid crystal phase. The reasons for using lecithin are: a) The peculiar feeling of lecithin on the skin. b) Skin familiarity - many materials make up the human skin cell wall. One of them is lecithin, a very important intercellular lipid. c) The formation of the lamella structure - the human skin is composed of lamella of lecithin system. When using lecithin, ceramide, and cholesterols altogether, lamella is easily formed, and artificial skins form around the outer walls of real skin. d) Increased moisturizing effect - compared to normal O/W and W/O systems, lamella structure has a high capacity of moisture keeping, owing to its peculiar structure. e) Improved skin penetration effects - by the skin familiarity, cosmetic ingredient - can penetrate skin more deeply. In this study, to measure skin barrier function bio-mimic liquid crystal emulsion (BLCE) was produced using higher fatty alcohol, lecithin, cholesterol and compared its cytotoxicity with general surfactants.

2. Materials and Methods

The lamellar structure is produced by using hydrogenated lecithin, batyl alcohol, cetostearyl alcohol, phytosterol and cholesterol. A very large amount of liquid crystal is observed on the emulsion as a result of polarized microscopic measurement in the phase transition of the mixed system. Hydrogenated lecithin, batyl alcohol, cetostearyl alcohol, phytosterol and cholesterol were mixed proportionally and cooled after having been heated to 90 ℃. Selected was the ratio when liquid crystals are observed most often.

3. Results

BLCE is stable as a result of inspection of its stability in a cycling incubator (-20 ℃ ~ 45 ℃) and Turbiscan for 6 weeks. The efficacy and functions of BLCE were studied by *in vitro* and *in vivo* test. It is also shown from the measurement of the skin barrier function by using Tewameter (CM 210, Courage & Khazaka, Germany) and Corneometer (TM 825, Courage & Khazaka, Germany) that the moisturization function

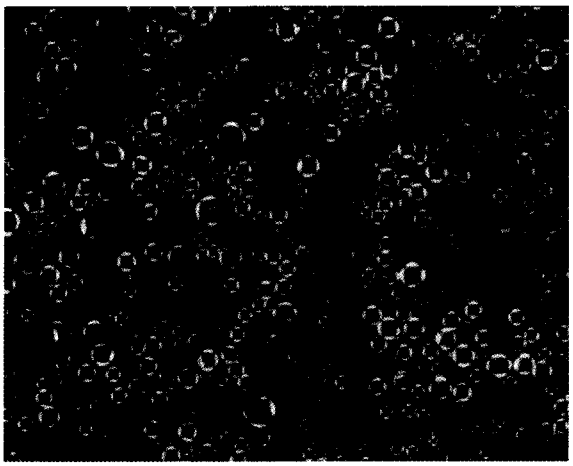

Figure 1. Optical microscopy photograph of BLCE (× 100, polarized light).

of the skin and restoration of the damaged skin are significantly improved. Furthermore, it is seen that irritation of BLCE is very small in view of the cytotoxicity when it is compared with those of other general surfactants.

3.1. Observing Liquid Crystal of BLCE

By using light a microscope (Bx50, OLYMPUS, Japan), optical anisotropic of Maltese cross and typical multilayers of emulsion was confirmed (Figure 1). The structures of those association are considered to be similar to the lamella structure of the horny layer.

3.2. Observation of the Stability of BLCE

The stability was observed by store BLCE into a cycling incubator (-20 ℃ ~ 45 ℃) for 6 weeks (Figure 2). As shown in the figure, the liquid crystal structure of the sample had not changed after 6 weeks when compared to the before-incubator status. The stability of BLCE was shown.

3.3. Moisturization Effect of BLCE and Measuring TEWL

To measure the moisturizing effect, Corneometer was used and to measure TEWL, Tewameter was used. Studies of the moisturizing effect of the emulsion were conducted on five female volunteers with healthy skin. The participants, aged 19 to 50, were briefed on the study procedures and each gave written informed consent. Measurements were carried out at 22 ± 1 ℃ and

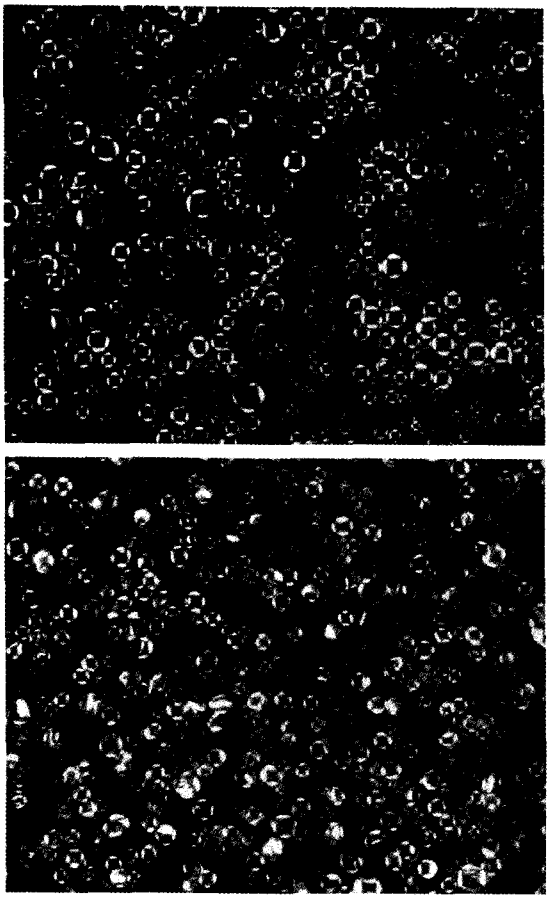

Figure 2. Optical microscopy photograph of BLCE (× 100, polarized light).

a relative humidity of 60 ± 10 %. Subjects adjusted to ambient conditions for 20 min prior to any measurement. The skin of the volar forearm was treated with a 5 % aqueous solution of sodium lauryl sulphate, and then covered with an occlusive dressing. Two hours later, the dressing was removed and the region was gently washed with water and air-dried. Base measurements were taken 30 min later. Then the five test products were applied at a dose of approximately 2 mg/cm^2, leaving one area untreated. Two daily home applications continued in the morning and evening over the next 14 days. Measurements were obtained during the treatment period on day 3, 7 and 14, one hour after the last daily application. Use of other cosmetic products was restricted on the test areas throughout the whole study period. The result of measurement is in the order of nanocare emulsion > general emulsion in the aspects of moisturization and TEWL (Figures 3, 4).

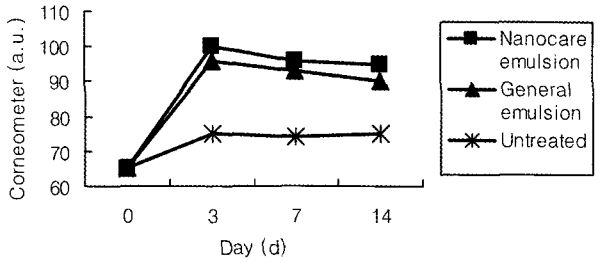

Figure 3. Effect of emulsion on forearm skin hydration.

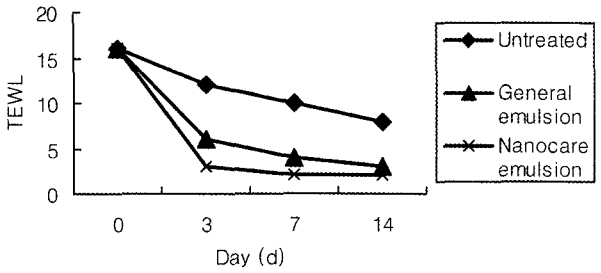

Figure 4. Effect of emulsion on forearm skin TEWL.

3.4. Cytotoxicity of Nanocare

The cytotoxicity, of nanocare, hydrogenated lecithin, sodium laureth sulphate and sorbitan stearate were measured. After diluting transformed mouse fibroblast L929 cells, obtained by typsinization were suspended in Dulbecco's modified Eagle's medium (DMEM) containing bovine calf serum (BCS) of 2 %. And after inoculating a suspension (2,500 ~ 3,000 cell/well) 90 μL onto each well of 96-well tissue culture plates, they were cultivated for 2 d at 37 ℃ under 5 % CO_2. After cultivation, new culture medium 90 μL substituted the old ones and cultivated for 2 d after treating 10 μL of test materials. After cultivation, to each well was added 100 μL of neutral red solution (50 μg/mL) for 3 h of activation. After treating living cells' lysosome with concentrated 1.0 % formalin/1.0 % $CaCl_2$ solution 100 μL by neutral red having passed through plasma membrane, neutral red in cells were extracted using 1.0 % acetic acid/50 % ethanol solution. The cytotoxicity of each surfactant was measured under 540 nm using ELISA reader on the extracted neutral red. After measurement, its toxicity was very low compared to normal surfactants (Figure 5).

4. Conclusion

In this study, after measuring in mixture status with

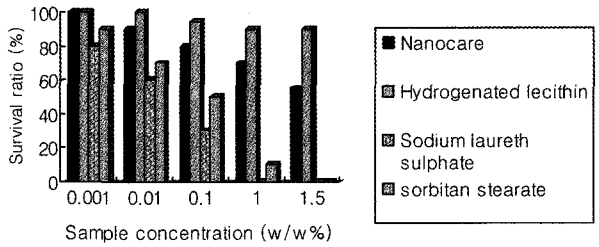

Figure 5. Cytotoxicity of surfactants.

Table 1. Production of Various Types of Emulsion

	Nanocare emulsion	General emulsion
Water	Make to 100	Make to 100
Glycerine	7	7
Nanocare	3	0
Tween 20	0	3.0
Tween 85	0	1.0
Preservation	0.3	0.3
Sun flower oil	10	10
Caprylic/capric triglycerides	5	5

a polarized microscope, much of lamella structure and liquid crystal in emulsion phase were observed using hydrogenated lecithin, batyl alcohol, cetostearyl alcohol, phytosterol and cholesterol. The stability of BLCE was proven in a cycling incubator (-20 ℃ ~ 45 ℃) for 6 weeks. After measuring using Tewameter and Corneometer to measure skin barrier function, the moisturization effect and improvement of damaged skin was considerably high. Furthermore, cytotoxicity was very low compared to normal surfactants.

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