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Anti-Blue Light, Anti-Pollution, and Anti-Wrinkle Effects of PrimeLipid™ PI: A Novel Bioactive Lipid-Ingredient, Phosphatidylinositol

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1. Introduction

We focused on phosphatidylinositol (PI) (Figure 1), a bioactive phospholipid, and developed a lipid complex, PrimeLipid PI, which contains a high content of PI. Although PI only accounts for a small (less than 10%) fraction of cellular phospholipids, it plays important roles in cell signaling, lipid signaling, and membrane trafficking. PrimeLipid PI is an easy-to-prepare liposomal preparation ingredient. It has moisturizing, anti-wrinkle, and antioxidant effects, which prevent the skin from harmful oxidation stress caused by blue light and air pollution. PrimeLipid PI is a new ingredient based on our innovative lipid technology.

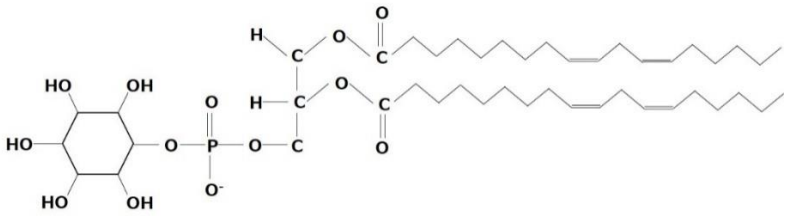


Figure 1. Representative structure of phosphatidylinositol (PI)

2-1. PrimeLipid™ PI

PrimeLipid PI is an ingredient that allows easy preparation of stable liposomes using a homomixer (Table 1). These liposomes contain a high content of phosphatidylinositol (PI) as a key ingredient and have a multilamellar structure (Fig. 2A). PrimeLipid PI-containing lotion has high transparency and stability (Fig. 2B).

Table. 1 Formulation composition of 0.2%(w/w) PrimeLipid PI lotion

Ingredients	%
PrimeLipid™ PI	0.2
Glycerin	3.0
Pentylene glycol	2.0
Phenoxyethanol	0.2
Water	Up to 100

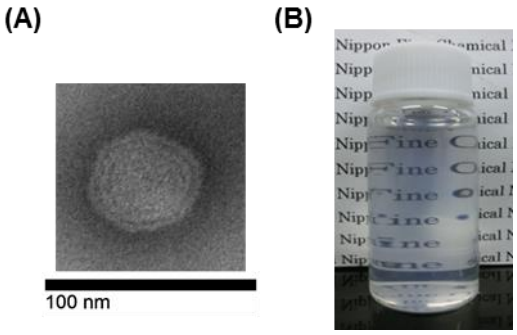


Figure. 2 PrimeLipid PI liposomes

(A) Transmission Electron Microscope (TEM) image of liposome prepared from PrimeLipid PI.
(B) Photograph of the PrimeLipid PI lotion.

2-2. Moisturizing effect

PrimeLipid PI lotion (Table. 1) was applied to the medial forearm of 11 male and female subjects (25 to 45 years of age) twice daily, and skin conductance was evaluated at weeks 0, 2, and 4 (Fig. 3). As a result, the skin conductance was improved by about 2 times in comparison with the untreated area.

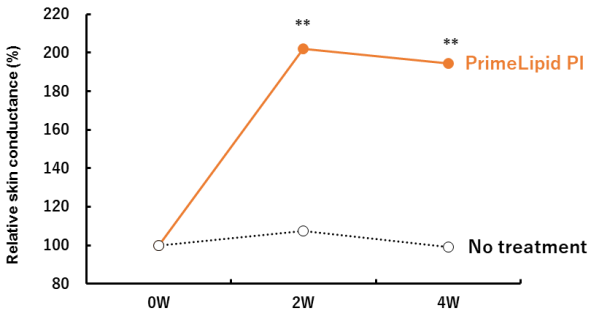


Figure 3. Changes in skin conductance following the application of PrimeLipid PI lotion

The water content of the stratum corneum layer was measured on days 0, 14, and 28 using a SKICON-200EX (Yayoi Co., Ltd.). Mean ± SD (N=11). ***P*<0.01 versus no treatment.

2-3. Anti-wrinkle effect

To examine the effect of the 0.2% PrimeLipid PI lotion (Table. 1), it was applied around one of the eyes of 18 female subjects (35 to 58 years of age) twice daily, and we found that the application improved the appearance of wrinkles on the outer corners of the eye. Representative photographs are shown in Figure 4A. The wrinkles on the outer corner of the eyes were less noticeable on the side treated with the PrimeLipid PI lotion compared to the untreated side. After 4 weeks, the wrinkle scale significantly improved

the sites treated with PrimeLipid PI lotion compared with the sites without treatment (Figure 4B).

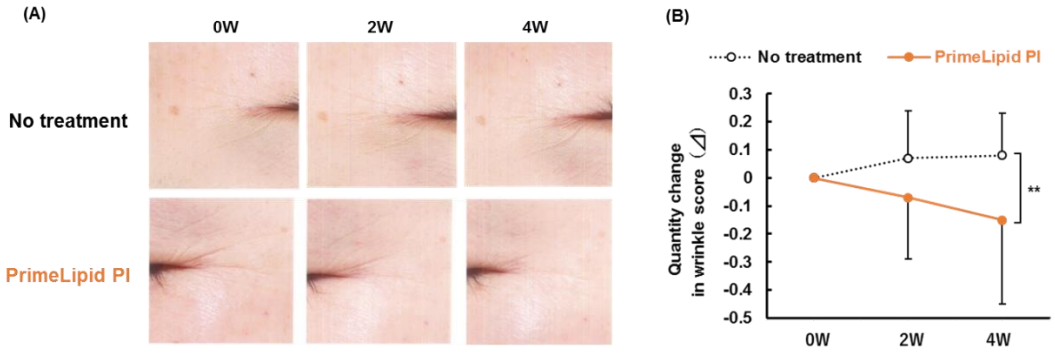


Figure 4. Improvement of wrinkles

(A) Representative photographs of wrinkles around the eyes after application of PrimeLipid PI lotion vs. no treatment after 0, 2, and 4 weeks. (B) Changes in wrinkle score after 0, 2, and 4 weeks of application of PrimeLipid PI (●) and after no treatment (O). Mean ± SD (n=18). Significantly different results: ***P*<0.01 (Wilcoxon signed-rank test).

2-4. Anti-oxidation effects

Oxidative stress (reactive oxygen species [ROS]) induced by chronic exposure to sunlight is considered to be one of the causes of cosmetic skin problems such as age spots and wrinkles. The Keap1-Nrf2 (Kelch ECH associating protein 1 – Nuclear factor erythroid 2-related factor 2) system is an innate protection system of cells from the harmful effects of ROS. Nrf2, which acts as a control tower for biological defense, moves into the nucleus when cells are exposed to oxidative stress, and induces the expression of many stress-responsive genes such as CAT (catalase) and γ-GCS (γ-glutamylcysteine synthetase)[1)].

PrimeLipid PI reduced intracellular ROS levels (Fig. 5A) by activating the Keap1-Nrf2 system. PrimeLipid PI treatment improved the nuclear translocation of Nrf2 in NHEKs (normal human epidermal keratinocytes), as observed by immunostaining (red: Nrf2, blue: nucleus, Fig. 5B). The Nrf2 and nuclear colocalization rate from the analysis of the acquired images had approximately a 2.5-fold increase in PrimeLipid PI-treated NHEKs (Fig. 5C). Prime Lipid PI elevated the gene expression of CAT, which scavenges hydrogen peroxide (Fig. 5D). Moreover, the catalase activity in the stratum corneum harvested by tape stripping from the skin at the site where the PrimeLipid PI lotion was applied for two weeks was approximately four times the one in the untreated

area (Fig. 5E). These results suggest that PrimeLipid PI is expected to maintain healthy skin the skin healthy by alleviating oxidative stress.

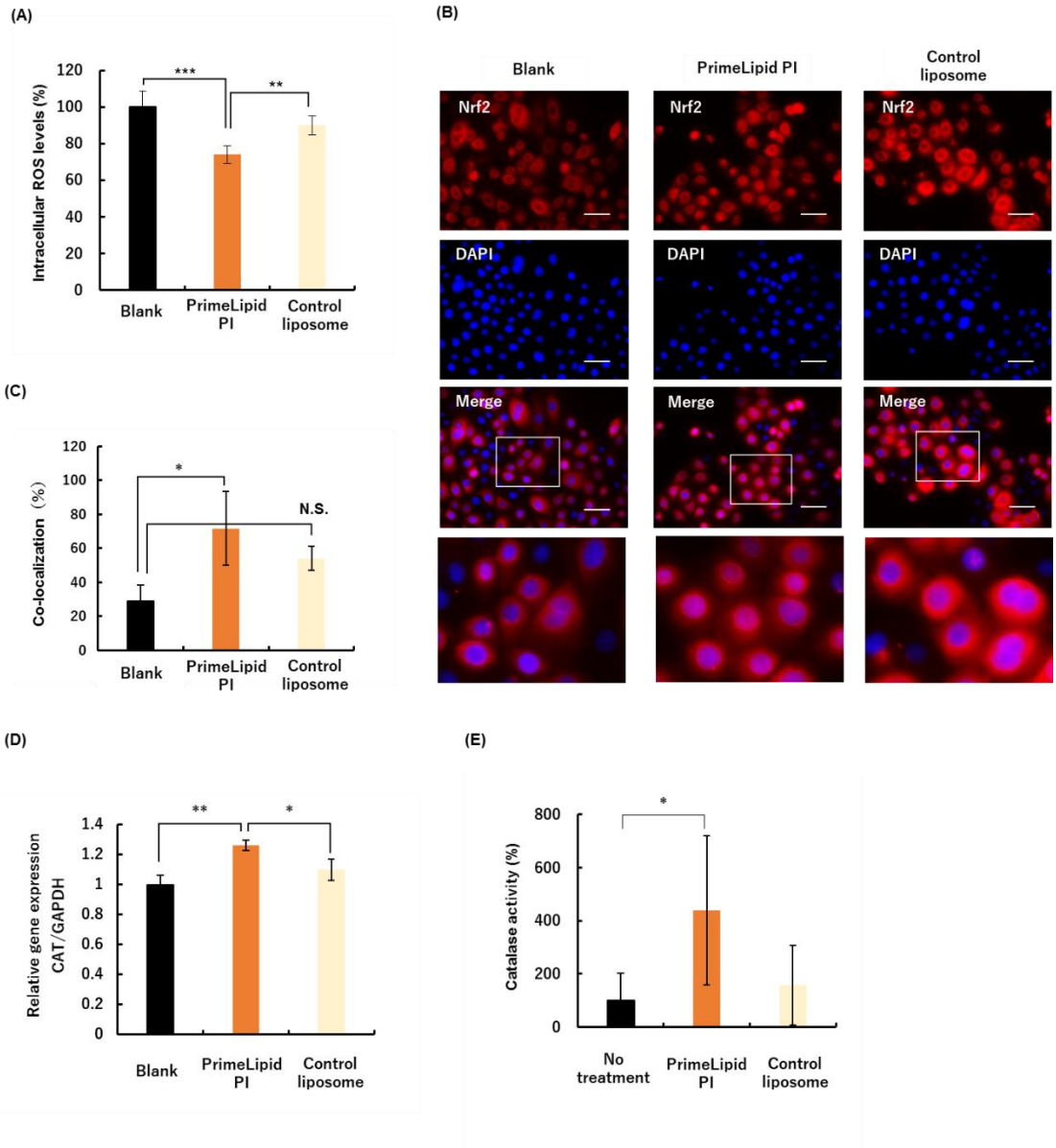


Figure 5. Anti-oxidant effects

(A) Intracellular ROS levels in NHEKs after 24 hours of incubation in PrimeLipid PI-containing medium. Mean ± SD (n=6). Significantly different results: ***P*<0.01, ****P*<0.001. (B) Nrf2 fluorescent immunostaining in NHEKs after 6 hours of incubation with PrimeLipid PI. Red: Nrf2, blue: DAPI. (C) Co-localization rate of Nrf2 and the nucleus. Mean ± S.D. Significantly different results: **P*<0.05. (D) CAT gene expression in NHEKs after 24n hours of incubation in PrimeLipid PI-containing medium. Mean ± SD (n=3). Significantly different results: **P*<0.05, ***P*<0.01. (E) Catalase activity in human stratum corneum harvested by tape stripping from the skin at the site

where PrimeLipid PI lotion was applied for two weeks vs. no treatment area. Mean ± SD (n=6). Significantly different results: *P<0.05.

2-5. Anti-Blue light effects

UV irradiation has been mentioned as one of the main causes of skin trouble until now. However, with the popularization of smartphones and LEDs, the effect of visible light, especially blue light (380-500 nm) with strong energy, on the skin is noticed. Activation of pigment cells and increase in ROS have been reported as effects of blue light on the skin[2), 3)]. In addition, mitochondria are strongly damaged by blue light because they have cytochrome c oxidase with an absorption peak at 440 nm and Flavin-containing oxidases with an absorption peak at 370 nm and 450 nm[4), 5)]. Since oxidative stress and mitochondrial dysfunction are factors causing aging, blue light may induce skin aging. Therefore, it is important to prevent oxidative stress due to blue light as well as UV irradiation for skin anti-aging.

PrimeLipid PI decreased intracellular (Fig. 6A) and mitochondrial ROS levels (Fig. 6B) in NHEKs exposed to 45 J/cm^2 of blue light. In addition, mitochondrial lipid peroxidation levels, which are caused by oxidative stress, were significantly decreased by the PrimeLipid PI treatment (Fig. 6C).

Daily exposure to blue light from digital devices, such as smartphones as well as the blue light contained in sunlight, may cause skin aging. In addition to protecting the skin using products such as sunscreen, the use of ingredients that exert their effects by acting from the inside of cells, such as PrimeLipid PI, is expected to lead to a total anti-aging approach.

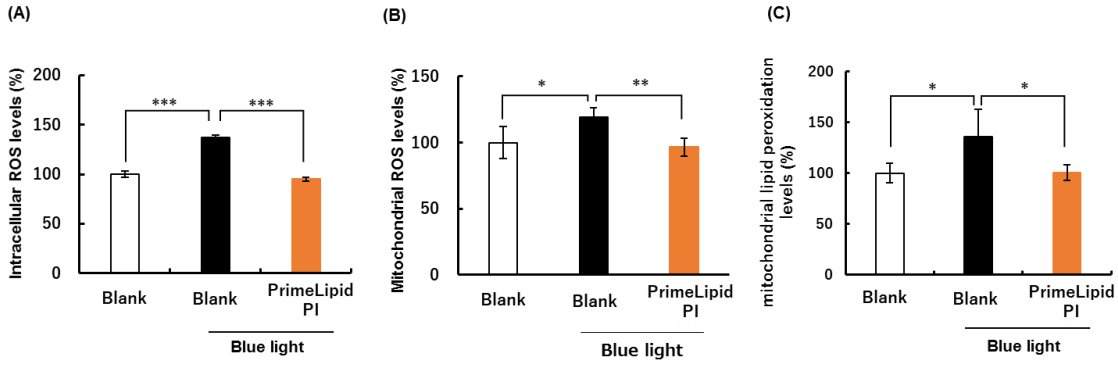


Figure 6. Anti-blue light effects

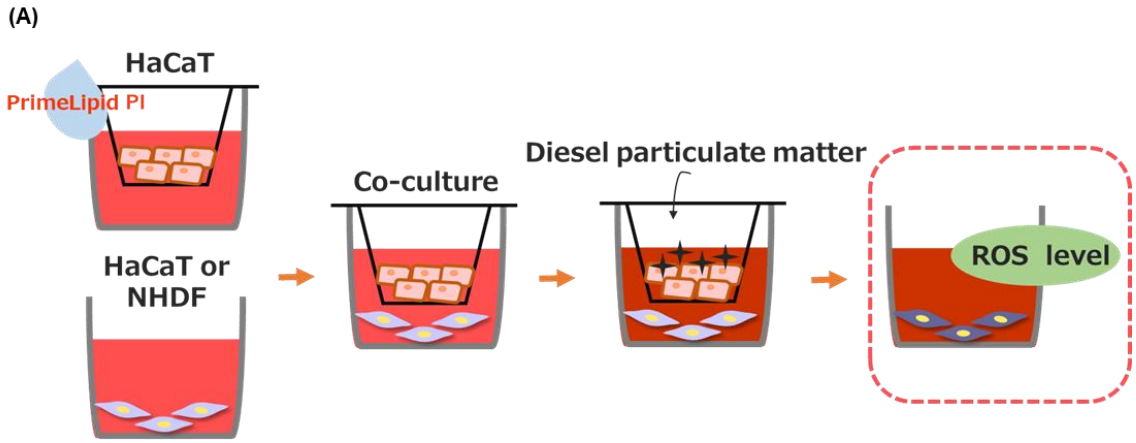
(A) Intracellular ROS levels in blue light-irradiated NHEKs, mean ± S.D. (n=6). Significantly different results: ***P<0.001. (B) Mitochondrial ROS levels in blue light-irradiated NHEKs, mean

± S.D. (n=4). Significantly different results: *P<0.05, **P<0.01 (C) Mitochondrial lipid peroxidation levels in blue light-irradiated NHEKs, mean ± S.D. (n=4). Significantly different results: *P<0.05.

2-7. Anti-pollution effects

Air pollutants such as particulate matter (PM) induce ROS in skin cells and are one of the external environmental factors that cause skin problems[6]. Epidemiological studies have shown that pigment spots and wrinkles are common among people living in areas with high levels of air pollutants, suggesting a direct association with skin aging [7]. We evaluated the intracellular ROS level as an index to confirm the anti-pollution effect of PrimeLipid PI using diesel particulate matter (DPM, NIST 1650b PM2. 5, Sigma-Aldrich) as air pollutants.

The human epidermal keratinocyte cell line (HaCaT) seeded in cell inserts was cultured in PrimeLipid PI-containing medium. After 24 h, HaCaT cells were co-incubated with freshly cultured HaCaT or human dermal fibroblasts (NHDFs) in the cell culture plate and exposed to DPM (200 μg/mL). After that, intracellular ROS levels in HaCaT and NHDFs, which were cultured in the cell culture plate, were evaluated (Fig. 7A). Intracellular ROS levels in HaCaT and NHDFs co-incubated with DPM-treated HaCaT cells were increased. These results indicate that PM causes oxidative stress in epidermal cells, and that this stress is transmitted to nearby keratinocytes and dermal fibroblasts. In contrast, HaCaT and NHDFs co-cultured with DPM and PrimeLipid PI-treated HaCaT cells showed no increase in intracellular ROS levels (Fig. 7B, C). PrimeLipid PI suppresses ROS in the skin cells induced by DPM and is expected to be effective anti-pollution cosmetic ingredient.



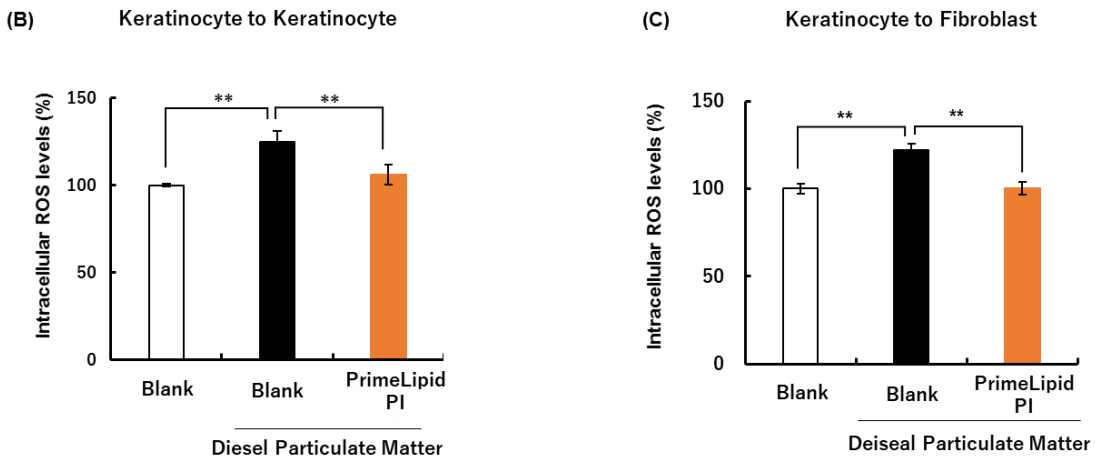


Figure 7. Anti-pollution effects

(A) Anti-pollution assay using a cell co-culture system (B) Intracellular ROS levels in HaCaT cells which was co-incubated with DPM-exposed HaCaT. (C) Intracellular ROS levels in NHDFs co-incubated with DPM-exposed HaCaT. Mean ± SD (n=4). Significantly different results: ***P*<0.01.

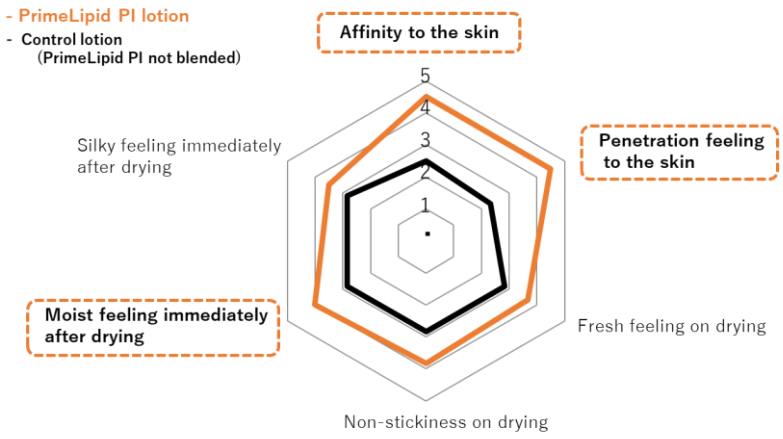


Figure 8. Sensory evaluation test

Results of the sensory evaluation tests using PrimeLipid PI lotion (Table. 1) and the control lotion from which PrimeLipid PI was removed (n=6).

3. Conclusion

PrimeLipid PI contains high concentrations of phosphatidylinositol (PI), which is a bioactive lipid. Although reports of the effect of PI on skin physiology have been scarce, our studies show that PrimeLipid PI has moisturizing, anti-wrinkle, antioxidant, and multi-physiological activity effects. Many studies have reported that various external factors such as blue light and air pollutants cause skin troubles[8]. PrimeLipid PI activates Nrf2,

an innate intracellular antioxidant system, and can prevent oxidative stress and skin aging. In addition, sensory evaluation tests showed that the PrimeLipid PI lotion (Table 1) improved the feeling of affinity, penetration feeling to the skin, and moist feeling immediately after drying (Fig. 8). PrimeLipid PI is a unique ingredient associated with multiple positive physiological activity effects on the skin and with good sensory feelings.

PrimeLipid™ is the registered trade mark of Nippon Fine Chemical Co., Ltd.

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