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Effects of Phosphoryl Oligosaccharides of Calcium (POs-Ca) on Epidermal Cells and Human Skin

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Haruyo Sambe,[1,†] Kazuhisa Sugimoto,[1] Koji Nomura,[1] and Takashi Kuriki[1]

[1]*Institute of Health Sciences, Ezaki Glico Co., Ltd.* (*4−6−5 Utajima, Nishiyodogawa-ku, Osaka 555−8502, Japan*)

Abstract: Phosphoryl oligosaccharides of calcium (POs-Ca) is a calcium salt of phosphoryl maltooligosaccharides made from potato starch. POs-Ca has high solubility in water and it can supply both calcium ion and acidic oligosaccharides in an aqueous medium. In this study, we evaluated effects of POs-Ca on cultured normal human epidermal keratinocytes (NHEK) and human skin. Several *in vitro* studies using cultured NHEK demonstrated that POs-Ca promoted NHEK differentiation, tight junction formation, intercellular lipid production, and gene expression involved in stratum corneum condition, skin barrier function and hydration. Skin penetration study using a three-dimensional epidermal model demonstrated that POs-Ca was able to provide both calcium ion and phosphoryl oligosaccharides to the epidermis. Furthermore, an *in vivo* study demonstrated that POs-Ca improved human skin conditions including hydration, barrier function, stratum corneum condition, and skin texture. These results suggest that POs-Ca can be a superior active agent for healthy epidermis.

Key words: phosphoryl oligosaccharides of calcium, calcium carrier, keratinocyte differentiation, tight junction, skin barrier, intercellular lipid

INTRODUCTION

Phosphoryl oligosaccharides of calcium (POs-Ca) is a calcium salt of phosphoryl oligosaccharides prepared from potato starch hydrolysate.[1−3)] The phosphoryl oligosaccharides are mainly composed of maltotriose, maltotetraose, and maltopentaose to which a phosphate group is bound. POs-Ca contains calcium approximately 5%. Generally, calcium phosphate has low solubility in water. However, POs-Ca has high solubility in water because it contains oligosaccharides that have extremely-high water solubility. Previous studies have demonstrated that POs-Ca has various functions such as mineral supplementation,[4)] and remineralization and recrystallization of tooth enamel lesions.[5−7)] Particularly, POs-Ca has already been put to practical use in chewing gum for prevention of dental caries.

We expect further application of POs-Ca as a superior water-soluble calcium compound. Calcium ion in the epidermis has key roles in the epidermal barrier homeostasis and repair[8,9)] such as terminal differentiation,[10)] formation of the cornified envelope,[11)] epidermal lipid synthesis[12)] and tight junction formation.[13)] The epidermis displays a characteristic calcium distribution so-called "epidermal calcium gradient", with high calcium levels in the outer stratum granulosum, tapering to very low levels in both the lower epidermis and stratum corneum (SC).[14)] The epidermal calcium gradient is associated with epidermal differentiation and barrier homeostasis,[15)] and it becomes broad with aging.[16)] Thus, calcium ion and its distribution in the epidermis are important for the epidermal function. Another interesting point is that phosphoryl oligosaccharides are anionic oligosaccharides. It has been reported that an external negative electric potential affects the epidermal ion gradient and accelerates skin barrier recovery after barrier disruption,[17)] and that anionic polymers accelerate the damaged skin barrier recovery.[18)] Moreover, a negative electric potential affects epidermal calcium distribution even in normal human skin.[19)]

POs-Ca can supply both calcium ion and anionic oligosaccharides in an aqueous medium. Therefore, we expect that POs-Ca will provide not only the calcium function in the epidermis but also negative electric potential on the skin surface, and POs-Ca will be useful for skin care. In this study, we evaluate the effects of POs-Ca on cultured epidermal keratinocytes and human skin.

MATERIALS AND METHODS

Materials. POs-Ca of cosmetic grade (Glico Nutrition Co., Ltd., Osaka, Japan) was used for all experiments. It contained phosphoryl maltotriose, phosphoryl maltotetraose, and phosphoryl maltopentaose as a major phosphoryl maltooligosaccharide,[20)] and 5.3% calcium. Analytical grade solvents and reagents were used for chromatography. Other reagents and materials were described in each experiment.

Cell culture. Normal human epidermal keratinocytes

† Corresponding author (Tel. +81−6−6477−8425, Fax. +81−6−6477−8362, E-mail: sambe-haruyo@glico.co.jp).

Abbreviations: POs-Ca, phosphoryl oligosaccharides of calcium; SC, stratum corneum; NHEK, normal human epidermal keratinocytes; GAPDH, glyceraldehydes-3-phosphate dehydrogenase; TER, trans-epithelial electric resistance; PPIA, peptidylprolyl isomerase A; OCDN, occludin; CLDN1, claudin 1; CLDN4, claudin 4; IVL, involucrin; TGM1, transglutaminase 1; KRT1, keratin 1; FLG, profilaggrin; HAS3, hyaluronan synthase 3; HPTLC, high-performance thin-layer chromatography; TEWL, trans-epidermal water loss.

(NHEK) were purchased from Kurabo Industries Ltd. (Osaka, Japan). NHEK were cultured in Gibco EpiLife medium with 0.06 mM calcium (Life Technologies Corporation, Carlsbad, USA) supplemented cell growth agents, KK-6150 (Kurabo) at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. At approximately 80% confluence, the cells were collected with trypsin, EDTA and HEPES buffer (Kurabo), and used in all experiments.

Morphological observation of NHEK. NHEK were seeded in a 96-well microculture plate at 1×10^4 cells/well and cultured for 24 h. The cultured medium was replaced by 200 μL of fresh one containing 0.05% POs-Ca, and the cells were cultured for 48 h. The morphology was observed under an inverted phase contrast light microscope.

Western blot analysis of involucrin in NHEK. NHEK were seeded in a 12-well microculture plate at 7×10^4 cells/well and cultured for 48 h. The cultured medium was replaced by 1 mL of fresh one containing 0.1% POs-Ca, and the cells were cultured for 144 h. Protein lysate of the cells was prepared using a cell disruption buffer of Ambion PARIS kit (Life Technologies). Protein concentration was determined with RC/DC Protein Assay (Bio-Rad Laboratories, Inc., Hercules, USA). Equal amounts of protein (1 μg) were separated by SDS-PAGE and transferred to nitrocellulose membrane and western blotting was performed for involucrin and glyceraldehydes-3-phosphate dehydrogenase (GAPDH) using ONE-HOUR Western Multiplex Kit I (GeneScript Inc., Piscataway, USA). Lab Vision anti-Involucrin Ab-1 (Thermo Fisher Scientific Inc., Waltham, USA) was used for primary antibody. GAPDH was used as an internal control. Densitometric analysis was performed using LAS-4000 UVmini (Fujifilm Corporation, Tokyo, Japan) and Multi Gauge version 3.0 (Fujifilm).

Measurements of transepithelial electric resistance. Tight junction forming ability was evaluated by measuring the transepithelial electric resistance (TER). TER was measured by a previously described method[21] using a Millicell-ERS epithelial volt ohmmeter (Millipore Corporation, Bedford, USA). NHEK were seeded onto Transwells (Millipore) of 0.4 μm pore size at 4×10^4 cells/well and cultured for 96 h. The culture medium was replaced by fresh one containing 0, 0.02, 0.06, and 0.1% POs-Ca, and then the cells were cultured for 72 h. The TER was measured at 0, 24, 48, and 72 h after the addition of POs-Ca.

Immunofluorescence microscopy. NHEK were seeded onto Transwells of 0.4 μm pore size at 4×10^4 cells/well and cultured for 24 h. The culture medium was replaced by fresh one containing 0.1% POs-Ca, and then the cells were cultured for 144 h. The cultured NHEK were fixed by 10% formalin, incubated with the primary antibodies, anti-claudin 1 antibody, anti-claudin 4 antibody and anti-occludin antibody, and thereafter incubated with fluorescent-conjugated secondary antibodies. The primary and secondary antibodies were purchased from Abcam plc. (Cambridge, UK). The images were obtained using a confocal laser scanning microscope.

RNA isolation and quantitative real-time PCR. NHEK were seeded in a 12-well microculture plate at 7×10^4 cells/well and cultured for 48 h. The culture medium was replaced by fresh one containing 0.1% POs-Ca, and then the cells were cultured for 0, 3, 12, and 24 h. Total RNA was isolated from the cells using RNeasy Mini Kit (Qiagen Inc., Valencia, USA) and cDNA was synthesized using Applied Biosystems High Capacity cDNA Reverse Transcription Kit (Life Technologies). Quantitative real-time PCR was performed on Applied Biosystems 7500 Fast Real-Time PCR System (Life Technologies) with gene-specific primer sets for occludin (OCDN), claudin 1 (CLDN1), claudin 4 (CLDN4), involucrin (IVL), transglutaminase 1 (TGM1), keratin 1 (KRT1), profilaggrin (FLG), and hyaluronan synthase 3 (HAS3), and Applied Biosystems Power SYBR Green PCR Master Mix (Life Technologies). Transcript levels were normalized to peptidylprolyl isomerase A (PPIA) gene. The primers were designed using Applied Biosystems Primer Express Software Version 3.0 (Life Technologies) and were synthesized by Sigma-Aldrich Corp. (St. Louis, USA). The primer sequences are as follows: PPIA-forward, 5′-GCTTTGGGTCCAGGAATGG-3′; PPIA-reverse, 5′-GTTGTCCACAGTCAGCAATGGT-3′; OCDN-forward, 5′-GCAGGAAGGTCAAAGAGAACAGA-3′; OCDN-reverse, 5′-GGACTCGCCGCCAGTTG-3′; CLDN1-forward, 5′-CTGGGAGGTGCCCTACTTTG-3′; CLDN1-reverse, 5′-CTTGGTGTTGGGTAAGAGGTTGT-3′; CLDN4-forward, 5′-GCTGGCCAGGATAGCTTAACC-3′; CLDN4-reverse, 5′-GCCAACGCCGATGCA-3′; IVL-forward, 5′-CCACTGGCTCCACTTATTTCG-3′; IVL-reverse, 5′-GGACAGACTCAAGTTCACAGATGAG-3′; TGM1-forward, 5′-GAGCGGAAGGCAGTAGAGACA-3′; TGM1-reverse, 5′-CCCCGGTTGGCATACACA-3′; KRT1-forward, 5′-CCAGGAGCTGATGAACACCAA-3′; KRT1-reverse, 5′-GAGGGTCCTGTAGGTGGCAAT-3′; FLG-forward, 5′-GGCACTGAAAGGCAAAAAGG-3′; FLG-reverse, 5′-AAACCCGATTCACCATAATCA-3′; HAS3-forward, 5′-GCCTATGTGACGGGCTACCA-3′; HAS3-reverse, 5′-ACAGGCCGAAGGACAGGTAGT-3′. PCR conditions are as follows: 95°C for 10 min, 40 cycles of 95°C for 15 s, and 60°C for 1 min. A dissociation stage was added after the PCR reaction to generate a melting curve for verification of amplification product specificity.

Analysis of intercellular lipid. NHEK were seeded in a 6-well microculture plate at 2×10^5 cells/well and cultured for 72 h. The culture medium was replaced by fresh one containing 0.1% POs-Ca, and then the cells were cultured for 144 h. Intercellular lipid in the NHEK was extracted using Bligh-Dyer method.[22] Separation of the extracted lipids was carried out by high-performance thin-layer chromatography (HPTLC) using a Silica Gel 60 HPTLC plate (Merck KGaA, Darmstadt, Germany) as previously reported.[23] After visualizing the spots in the HPTLC plate, the amounts of ceramides and cholesterol were quantitatively determined using LAS-4000 UVmini and Multi Gauge version 3.0. Standards of ceramide II, ceramide III, and ceramide VI were provided from Nikko Chemicals Co., Ltd. (Tokyo, Japan).

Skin penetration study. Three-dimensional cultured human epidermal model with advenced barrier function, EpiDerm EPI-200-X (MatTek Corporation, Ashland, USA), was set to the fixture, EPI-100-FIX (MatTek), and put in a 6-well plate added 5 mL of aqueous solution of 10 mM Tris buffer (pH 7.0)−0.9% NaCl as a receiver solution. Aqueous solution of 2% POs-Ca dissolved in 10 mM Tris buffer (pH 7.0)−0.9% NaCl was used as a donor solution. Donor solution (100 μL) was added onto the SC of the epidermal model. After 24-hour incubation at 37°C, the receiver solution and epidermal

model samples were recovered. Surface of the epidermal model was washed sufficiently using 10 mM Tris buffer (pH 7.0)−0.9% NaCl and the whole model was homogenated in 0.5 mL of 10 mM sodium acetate buffer (pH 5.5). Phosphoryl oligosaccharides and its major metabolites, glucose and maltooligosaccharides in those samples were analyzed by ion-exchange high performance liquid chromatography with Dionex CarboPac PA-100 anion-exchange column (Thermo Fisher Scientific) under conditions as previously reported.[1] Calcium ion in the samples was analyzed by ion-exchange high performance liquid chromatography with Dionex IonPac CS16 cation-exchange column (Thermo Fisher Scientific) under usual conditions using 30 mM methanesulfonic acid as an eluent.

In vivo study. The *in vivo* effect of POs-Ca was evaluated in an 8-week open study with 2% POs-Ca aqueous solution containing 0.2% methyl paraben. Twenty-one healthy female subjects, between 22 and 52 years old who suffered from rough skin, applied the test sample to the face just before daily skin care in the morning and at night, twice a day for 8 weeks. SC hydration,[24] trans-epidermal water loss (TEWL),[24] parameters of SC condition and skin texture were measured before the application and after a 4- and 8-week application. SC hydration and TEWL were measured with Corneometer CM 825 (Courage + Khazaka Electronic GmbH, Cologne, Germany) and VapoMeter SWL-4001TJ (Delfin Technology Oy, Kuopio, Finland), respectively. SC condition was evaluated by imaging analysis of tape-stripped SC cells.[25] Skin texture was evaluated by imaging analysis of silicone skin replica.[24] The test was carried out from January to March in Japan. Pollen allergy sufferers were excluded at the selection stage of subjects. All subjects were asked to following things: i) do not shave measurement site on face, ii) do not change basic cosmetics, iii) do not lead an irregular life. This study was conducted in accordance with Declaration of Helsinki, and was approved by Shiba Palace Clinic institutional review board (Tokyo, Japan).

Statistical analysis. Results are expressed as mean ± SD or mean ± SE. Statistical significance was determined by Welch's *t* test or Dunnett's multiple comparison test.

RESULTS AND DISCUSSION

Effect of POs-Ca on NHEK differentiation.

We examined the effects of POs-Ca on morphological property of NHEK and cell differentiation. In NHEK cultured in the presence of 0.05% POs-Ca, flattening and stratification of cells, which are characteristics of differentiated keratinocytes, were observed (Fig. S1; see *J. Appl. Glycosci.* Web site). Figure 1 shows the production of involucrin protein, a keratinocyte differentiation marker, in 0.1% POs-Ca treated and untreated cells. Involucrin production of POs-Ca treated group increased 1.7-fold compared to the untreated group. These results suggest that POs-Ca promotes NHEK differentiation. We confirmed that calcium of POs-Ca effectively acted on NHEK.

Effect of POs-Ca on tight junction formation of NHEK.

Tight junction is one of the intercellular junctions and forms a diffusion barrier that prevents the passage of molecules and ions through the space between cells. Tight junction in the epidermis is known to play an important role in maintaining the epidermal barrier function.[13] Calcium is deeply-involved in tight junction formation.

We examined the effect of POs-Ca on tight junction formation of NHEK. The degree of the sealing of tight junction was evaluated by measuring TER. TER of a keratinocyte sheet reflects the transepithelial permeability of water-soluble ions, and a higher TER indicates a lower ionic permeability. As shown in Fig. 2, POs-Ca markedly increased TER in a concentration-dependent manner. Next, immunostaining of tight junction proteins, occludin, and claudins, was performed using NHEK cultured with and without POs-Ca, respectively. Continuous and honeycomb localization of the tight junction proteins at cell borders, which is a typical image of tight junction formation, were observed only in POs-Ca treated cells (Fig. S2; see *J. Appl. Glycosci.* Web site). On the other hand, the localization of those proteins was not detected in the untreated cells (Fig. S2; see *J. Appl. Glycosci.* Web site). Furthermore, the gene expression of occludin and claudins was examined. As shown in Fig. 3, the expression levels of those genes were significantly increased by the treatment with 0.1% POs-Ca.

These results suggest that POs-Ca promotes tight junction formation of NHEK and that the promoting effect is

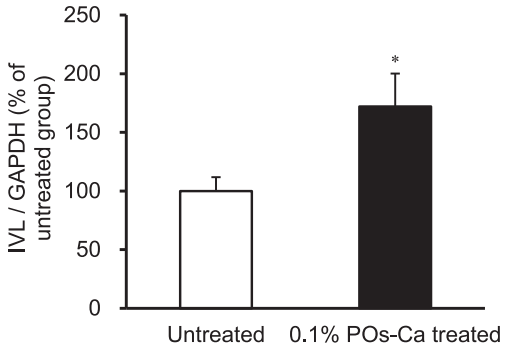

Fig. 1. Effect of POs-Ca on production of involucrin protein in cultured NHEK.

NHEK were treated and untreated with 0.1% POs-Ca for 144 h. Involucrin was a NHEK differentiation marker and the protein levels were normalized to GAPDH protein levels. Values are presented as mean ± SD (n = 3). Statistical analysis by Welch's *t* test, $^*p < 0.05$ *versus* untreated group.

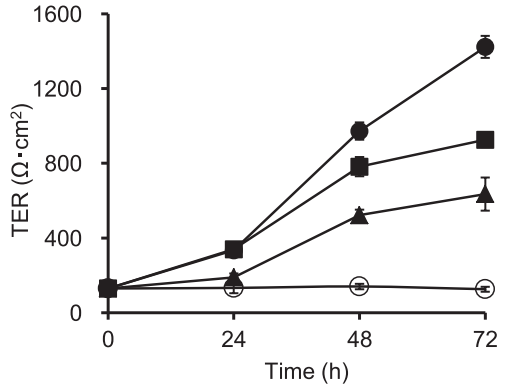

Fig. 2. Effect of POs-Ca on tight junction forming ability of cultured NHEK.

NHEK were treated with 0% (○), 0.02% (▲), 0.06% (■), and 0.1% (●) POs-Ca for 72 h. Tight junction forming ability was evaluated by measuring the TER. Values are presented as mean ± SD (n = 3).

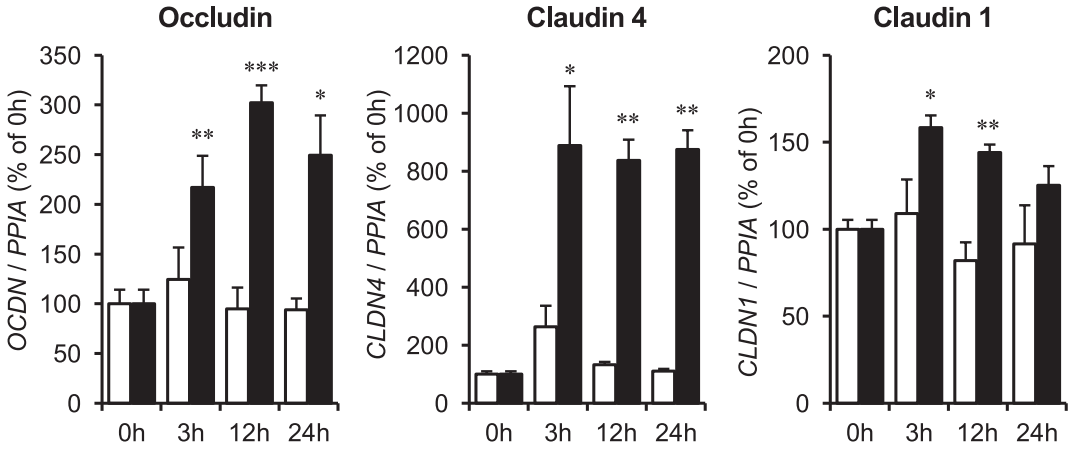

Fig. 3. Effect of POs-Ca on gene expression of tight junction related proteins in cultured NHEK.

NHEK were treated (■) and untreated (□) with 0.1% POs-Ca for 0, 3, 12, and 24 h. Transcript levels were normalized to *PPIA*. Values are presented as mean ± SD ($n = 3$). Statistical analysis by Welch's *t* test, $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ *versus* untreated group at the same incubation time.

controlled by transcriptional regulation and functional localization of tight junction related molecules.

Effect of POs-Ca on intercellular lipid production in NHEK.

Intercellular lipid is essential for SC barrier on the outermost layer of epidermis, and contains ceramides, cholesterol, and fatty acids as major components.

We examined the effect of POs-Ca on the production of intercellular lipid in cultured NHEK. Figure 4 shows the production of cholesterol and ceramides in POs-Ca treated and untreated groups. Cholesterol, ceramide II, and ceramide VI were significantly increased by the treatment of 0.1% POs-Ca. The spots of ceramide III in both groups were too weak to quantify. With the visual observation of the HPTLC plates, a small amount of ceramide III was detected in POs-Ca treated group, whereas no ceramide III was detected in the untreated group. These results suggest that POs-Ca promotes the production of intercellular lipid in cultured NHEK, and can be expected to enhance SC barrier.

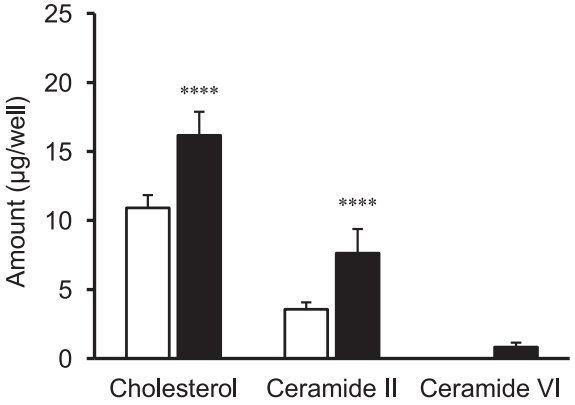

Fig. 4. Effect of POs-Ca on production of intercellular lipid in cultured NHEK.

NHEK were treated (■) and untreated (□) with 0.1% POs-Ca for 144 h. Intercellular lipid in cultured NHEK was extracted using Bligh-Dyer method and the amounts of cholesterol and ceramides were determined by HPTLC. Values are presented as mean ± SD ($n = 9$). Statistical analysis by Welch's *t* test, $^{****}p < 0.0001$ *versus* untreated group at the same incubation time.

Effect of POs-Ca on gene expression related to SC condition, barrier function and skin hydration in NHEK.

We examined the effect of POs-Ca on the expression of various genes involved in SC condition, barrier function and skin hydration (IVL, TGM1, KRT1, FLG, HAS3). As shown in Fig. 5, the expression levels of these genes were significantly increased by the treatment with 0.1% POs-Ca. This result suggests that POs-Ca can be expected to improve SC condition and skin hydration.

Skin penetration study of POs-Ca using three-dimensional cultured human epidermal model.

We evaluated skin penetration of POs-Ca using a three-dimensional cultured epidermal model with advanced barrier function which consists of organized basal, spinous, granular, and cornified layers analogous to those found *in vivo*. Table 1 shows calcium ion concentration in the receiver solution and epidermal model treated with 2% POs-Ca for 24 h. The background level was detected in the receiver solution samples of POs-Ca treated and untreated groups, whereas the concentration in the epidermal model of POs-Ca treated group was increased 3.6-fold compared to that in the epidermal model of untreated group. In addition, the estimated calcium ion concentration in the epidermal model was close to the effective concentration on cultured NHEK. Phosphoryl oligosaccharides were not detected in the receiver solution samples of both groups, whereas phosphoryl oligosaccharides and its metabolites were detected in the epidermal model sample of POs-Ca treated group (data not shown). In the extract of the epidermal model treated with POs-Ca, not only phosphoryl maltotriose with MW 616, one of the major phosphoryl oligosaccharides of POs-Ca, but also phosphoryl oligosaccharides with larger molecular weight, phosphoryl maltotetraose (MW 778) and phosphoryl maltopentaose (MW 940), were detected. Glucose, maltose, maltotriose, maltotetraose, and maltopentaose were detected as metabolites of POs-Ca.

From these results, we confirmed that calcium and phosphoryl oligosaccharides in POs-Ca was able to penetrate into the epidermis of epidermal model. We expect that POs-Ca will provide a suitable amount of calcium ion to the epidermis and also give anionic oligosaccharides to the

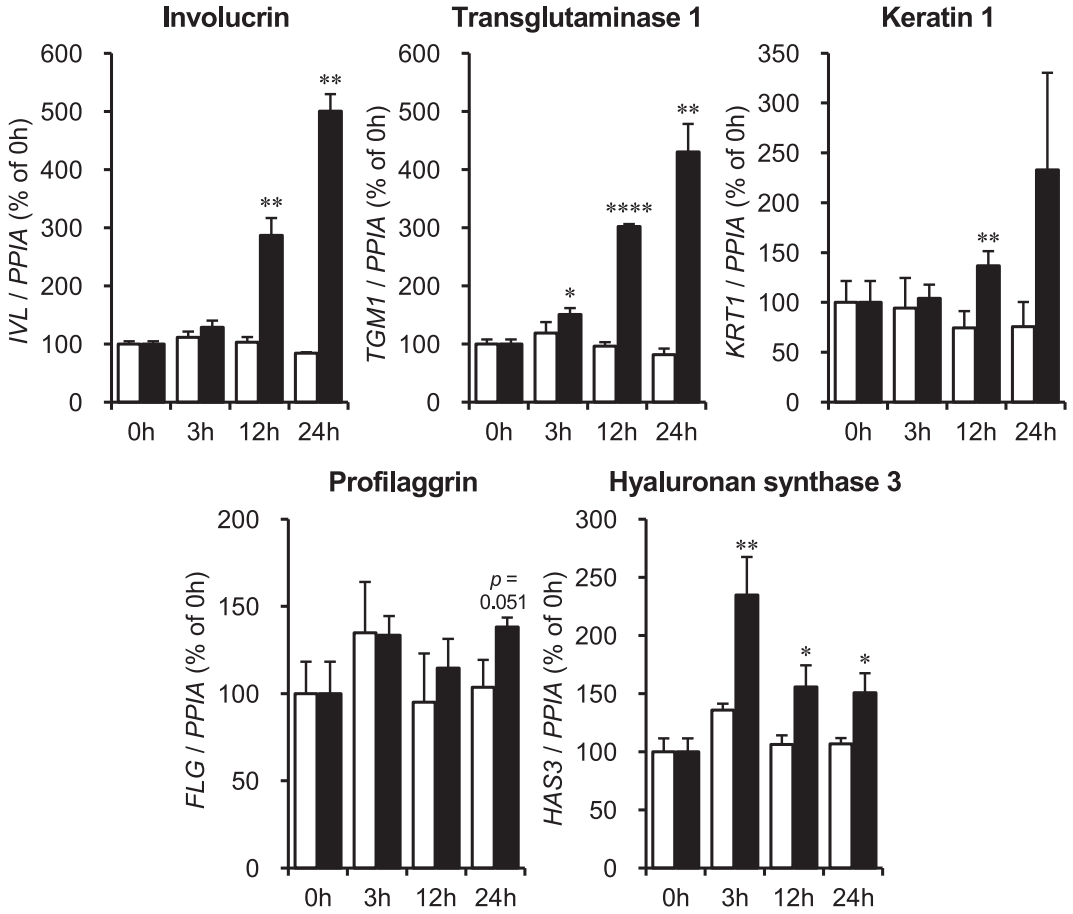

Fig. 5. Effect of POs-Ca on gene expression related to SC condition, barrier function and skin hydration.

NHEK were treated (■) and untreated (□) with 0.1% POs-Ca for 0, 3, 12, and 24 h. Transcript levels were normalized to *PPIA*. Values are presented as mean ± SD ($n = 3$). Statistical analysis by Welch's *t* test, $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{****}p < 0.0001$ *versus* untreated group at the same incubation time.

Table 1. Calcium ion (Ca^{2+}) concentration in receiver solution and epidermal model on skin penetration study of POs-Ca.

	Ca^{2+} concentration (mM)	
	Receiver solution	Epidermal model
POs-Ca treated group	Background level	0.69 ± 0.12*
Untreated group	Background level	0.19 ± 0.02

The values of epidermal model were calculated with approximate moisture content of epidermal model, 70%, and are presented as mean ± SD ($n = 3$). Statistical analysis by Welch's *t* test, $^{*}p < 0.05$ *versus* untreated group.

epidermis and the skin surface. In addition, we expect that phosphoryl oligosaccharides will act as a moisturizer at both epidermis and skin surface.

In vivo study.

Several *in vitro* studies suggest that POs-Ca has useful effects for cultured NHEK and it can penetrate into the epidermis. Next, we tried an *in vivo* study of POs-Ca on improvement of skin conditions. The skin parameters measured after 4- and 8-week application of 2% POs-Ca were compared before the application. The results were summarized in Fig. 6.

TEWL is a parameter for evaluating skin barrier function. After 4- and 8-week application of 2% POs-Ca, TEWL significantly decreased (Fig. 6(a)) and SC hydration significantly increased (Fig. 6(b)). These results suggest that POs-Ca has good moisturizing effect. The rate of multilayered SC cells is a relative proportion of piled-up SC removed by tape-stripping. Single-layered cells desquamate in normal skin, whereas multilayered cells desquamate in dry skin and/or aged skin. In an imaging analysis of the tape-stripped SC cells, a significant decrease in the rate of multilayered desquamation of SC cells was observed (Fig. 6(c)). This result suggests that POs-Ca improves desquamation function of SC cells. The area of the tape-stripped SC cells increased in subjects in their 20s and significantly decreased in subjects in their 40s to 50s (Fig. 6(d)). With the exception of the area of the tape-stripped SC cells, the results in each age group showed similar tendency as those in the whole group (data not shown). It is known that the area of SC cells is correlated with the epidermal turnover time and age.[25] We think that POs-Ca could contribute to the conditioning of SC and regulation of epidermal turnover. Results of the imaging analysis of silicone skin replica (Fig. 6(e)–(g)) showed that the skin texture significantly became finer and smoother. We found that POs-Ca was also effective in improving the skin texture.

From these results of the *in vivo* study, we confirmed that transdermal application of POs-Ca was effective for improvement of skin conditions including skin barrier function, hydration, SC function, and skin texture. It has been reported that the calcium gradient in normal, young epidermis becomes shallower with aging,[16] and that an external negative electric potential affects epidermal ion distribution and skin barrier homeostasis.[17–19] We think that

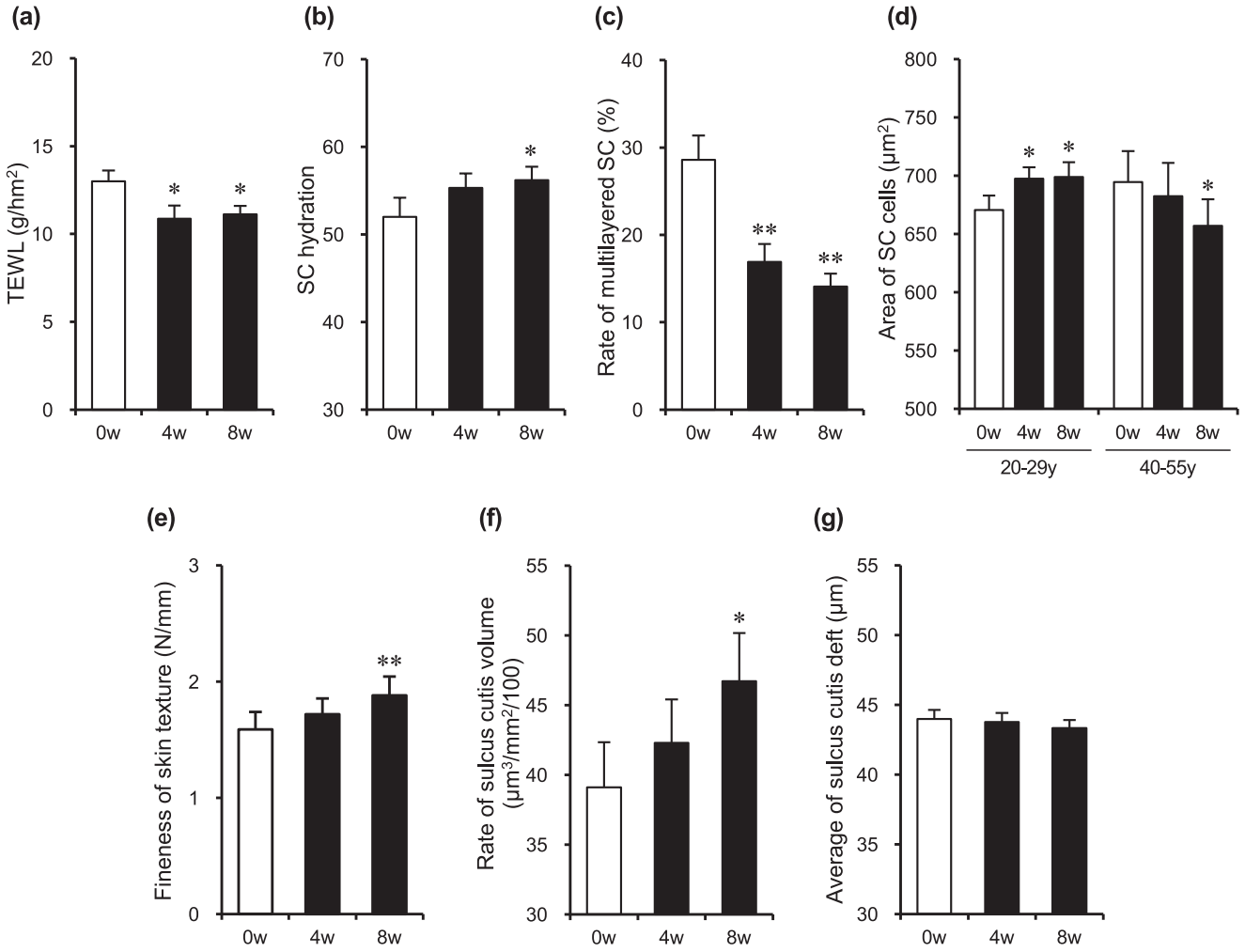

Fig. 6. *In vivo* effects of POs-Ca on skin barrier function (a), SC hydration (b), SC condition (c, d) and skin texture (e, f, g), after 4-week (4w) and 8-week (8w) application.

Aqueous solution of 2% POs-Ca was applied to the face just before daily skin care (morning, night) every day for 8 weeks. Values are presented as mean ± SE ($n = 21$: 20−29 years old, 10 female subjects; 40−55 years old, 11 female subjects). Statistical analysis by Dunnett's multiple comparisons test, $^{*}p < 0.05$ and $^{**}p < 0.01$ *versus* before the application (0w).

POs-Ca could be normalize the disordered calcium gradient by providing water soluble calcium and/or anionic oligosaccharides. Another previous study demonstrated that the effect of the application of a mixture of calcium and magnesium salt on skin barrier homeostasis was higher than that of each of these salts.[26] The report suggested that the effects of these metal ions were different depending on the counter ion and/or the method of application and that the difference of hydration level of them might preserve a better condition for skin barrier homeostasis. Our study suggests that the combination of the phosphoryl oligosaccharides ions and calcium ion might be a beneficial method of application for skin barrier homeostasis.

In vitro and *in vivo* studies suggest that POs-Ca can be useful to improve epidermal functions such as skin barrier function and moisturization. In conclusion, POs-Ca has the potential to be a superior active agent for healthy epidermis.

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