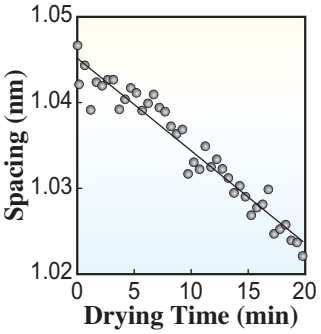
Moisturizing mechanism of glycerol and diglycerol on human stratum corneum

With regard to the moisturizing mechanism of glycerol and diglycerol, we found that there are two essential structural elements in the stratum corneum (SC). One is soft keratin in corneocytes, and the other is a domain formed by the orthorhombic hydrocarbon-chain packing (ORTHO) and the short lamellar structure (SLS). The former functions in storing water in the corneocytes, and the latter functions in regulating the water content of the corneocytes [1]. It is, however, difficult to detect the structural changes caused by these moisturizers without using synchrotron X-ray diffraction. In this study, we found that glycerol enhances both functions and that diglycerol negligibly affects soft keratin but greatly enhances water regulation [2]. Until now, the widely accepted result of a study on a lipid model system containing phospholipids, that is, glycerol has a significant effect on the appearance of a large amount of the liquid state, has led to the assumption that it provides the moisturizing effect [3]. In the present study using human SC we found that this is not the case in the moisturizing mechanism of glycerol. The X-ray diffraction experiments were performed at SPring-8 **BL40B2** and **BL19B2**. We measured the dynamic structural changes during the drying process under dry N_2 gas flow in SC samples treated with water or with aqueous solutions of polyol, including glycerol, diglycerol, or glycerol/ diglycerol.

In Fig. 1, the results for soft keratin in the corneocytes are summarized. Soft keratin is constructed of a rigid core and a terminal flexible segment. We can determine the inter-α-helix distance (approximately 1 nm) in a coiled-coil α-helix in the rigid core by X-ray diffraction measurement [1]. The relationship between the structures of the rigid core and the flexible terminal segment, which is directly affected by the polyols, is discussed later. The left side of Fig. 1 shows the change in the inter-α-helix distance in soft keratin during the process of drying a SC sample treated with water. The inter-α-helix distance decreased when the SC sample was dried. This is consistent with the result obtained as a function of the water content in static X-ray diffraction experiments [1]. The right side of Fig. 1 shows the decreasing rates of the inter-α-helix distance in the SC treated with water and the polyol aqueous solutions. The decreasing rates are characterized by two categories. The decreasing slope is steep in the SC treated with water and diglycerol but relatively gentle



Solution	Decreasing slope (nm/min)
water	0.0011
10wt% Diglycerol	0.0009
10wt% Glycerol	0.0004
7wt% Glycerol / 3wt% Diglycerol	0.0002

Fig. 1. Temporal change of the inter-α-helix distance in the rigid core of soft keratin during process of drying the stratum corneum sample treated with water. The table on the right side shows the decreasing slope obtained in the stratum corneum samples treated with water and polyol aqueous solutions.

in the SC treated with glycerol and glycerol/diglycerol. Our results indicate that in the former, diglycerol hardly penetrates into the corneocytes since the SC treated with diglycerol aqueous solution shows a similar effect to that treated with water, but in the latter, the glycerol component penetrates into the corneocytes; therefore, the effect of glycerol lasts longer.

We consider the role of glycerol in soft keratin. As shown by a nuclear magnetic resonance study on the SC [4], natural moisturizing factor (NMF) combine with the flexible segment of soft keratin and, as a result, attract water. Importantly, the study showed the change in the mobility of the flexible terminal segment strongly affects the mobility in the rigid core. In our study, we observed that the inter-α-helix distance in the rigid core decreases relatively gently in the process of drying the SC sample treated with glycerol. This suggests that glycerol can be retained in the flexible segment more strongly than NMF and bound water around glycerol is stored for a long time (Fig. 2). The role of diglycerol will be described later.

The intercellular lipids present around the corneocytes form the lamellar structures. The SLS has water layers and regulates water in the SC [1]. We observed the changes in the spacing of the ORTHO (approximately 0.42 and 0.37 nm). These spacing changes of 0.42 and 0.37 nm were not significantly different. Therefore, we show the changes in the spacing at 0.42 nm as a representative. The ORTHO and the SLS are in the common domain. Since the

X-ray diffraction peak for the spacing at 0.42 nm was narrow and strong, we were able to detect the slight change of the peak in the synchrotron X-ray diffraction experiment, notwithstanding that the change due to hydration and dehydration was only about 0.1%. The left side of Fig. 3 shows the change of the 0.42 nm spacing during the process of drying the SC sample treated with water. The spacing increased during wetting, whereas during drying it exhibited a complex behavior. During the drying process, first the spacing decreased rapidly, became nearly flat at the minimum, turned to increase, and finally remained almost unchanged. In the interval where the spacing was minimum, the peak became sharp, that is, the full width at half-maximum became narrow. This finding indicates that the domain of the ORTHO/SLS is in a stable state, where the water content may be about 25 wt%. A similar complex behavior was seen in the polyol aqueous solutions, but the rapid changes at transients became slightly broad. The right side of Fig. 3 shows the time for which the stable state was maintained, including transient intervals. The time is longer when samples are treated with polyol aqueous solutions than with water. These results suggest that the polyols stabilize the soft keratin structure that is related to the function of regulating water in the SC. It can be presumed that these retention times prolonged by the polyols are due to the interaction between the hydroxyl group of the polyols and the hydrophilic head group of intercellular lipids. It might be interpreted that diglycerol interacts with many intercellular lipid head groups, since diglycerol has a larger molecular length than glycerol. As a result, it is speculated that the polyols act on the intercellular lipid structure and the hydrocarbon chains become packed. This contradicts the results of the study by Froebe *et al.* on a lipid model, in which glycerol was said to transform the intercellular lipid structure to the liquid state, that is, glycerol produces the disordered structure [3]. The contradiction seems to lie in the fact that they used the lipid model containing phospholipids, which showed a rather different behavior from that of the intercellular lipids in the SC.

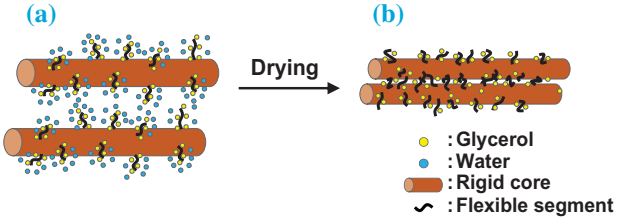


Fig. 2. Structure of soft keratin under two conditions. **(a)** Glycerol was applied to the stratum corneum. **(b)** The glycerol-applied stratum corneum was dried.

In conclusion, the polyols enhance the moisturizing function of the SC; specifically, in addition to the role of the NMF, glycerol acts on soft keratin in the corneocytes to reinforce water storage, and diglycerol acts on intercellular lipids to reinforce the ability of SC to regulate the water content. Therefore, it is highly possible that the combination of glycerol and diglycerol improved the moisturizing effect synergistically, and the water content of the SC could be retained for a long time under a dry condition. This is supported by the result of our previous study that the increase in the water content of the SC treated with the glycerol and diglycerol solution could be measured by electrical capacitance [5]. Cosmetics formulated with both glycerol and diglycerol would provide an enhanced moisturizing effect, that can be presumed to maintain healthy skin, of which 25 wt% of its water content is at the surface. The combined effect of glycerol and diglycerol revealed by the present scientific evidence has attracted attention in the cosmetic industry and has become popular. Nowadays, cosmetics formulated with glycerol and diglycerol are distributed worldwide.

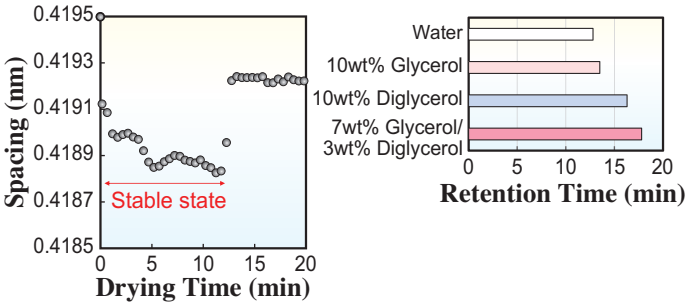


Fig. 3. Temporal change of the spacing of the orthorhombic hydrocarbon-chain packing structure during process of drying the stratum corneum sample treated with water. The right side shows the retention time of the stable state.

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