Lipid structure in stratum corneum of human skin

The top layer of human skin, stratum corneum (SC), serves as a barrier which protects human body from dehydration and penetration of unwanted substances. SC comprises of inactive cells (corneocytes) in which keratin filaments are densely packed and lipids such as ceramide, cholesterol, fatty acids and glycerides fill the space between the cells (brick-mortar model, Fig. 1). The barrier function is considered to mostly depend on the lipids that fill the gap of less than 50 nm wide. Many studies on the intercellular lipid structure have been carried out using electron microscopy, electron diffraction and X-ray diffraction. However, in most experiments SC was chemically treated to prepare specimens suitable for each technique. Therefore, we performed small-angle X-ray diffraction (SAXD) measurements of SC on intact human skin to examine structure of SC in our body [1].

Human skin, obtained in a cosmetic surgery and provided by a supplier, was folded to form a pointed edge. An X-ray microbeam with a diameter of approximately 5 μm [2], obtained with a pinhole at SPring-8 **BL40XU**, was passed at the edge to obtain diffraction from SC so that we could study skin with X-rays under conditions as physiological as possible. The skin was moved so that diffraction patterns from different depths were recorded.

The diffraction pattern in Fig. 2(a) shows three major peaks at $q = 0.51$, 1.03 and 1.4 nm^{-1} ($q = (4\pi/\lambda)\sin(2\theta/2)$ where 2θ is the scattering angle). In most of the typical results obtained in this study, the peaks at $q = 1.03$ and 1.4 nm^{-1} are much stronger than that at $q = 0.51$ nm^{-1} in the region close to the skin surface, while the 0.51 nm^{-1} peak becomes stronger in deeper regions (Fig. 2(b)). When measured at the depth where the peaks are strongest, the ratio of the spacings of the two peaks is close to 2, suggesting that these are the first and second orders of the 12.3 nm periodicity (periodicity d is obtained by $d = 2\pi/q$). Additionally,

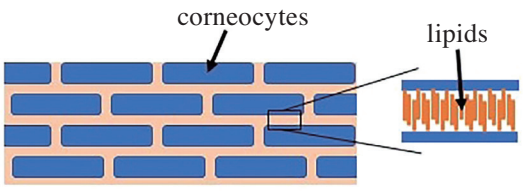

Fig. 1. The so-called brick-and-mortar model of stratum corneum which is 10–20 μm in thickness.

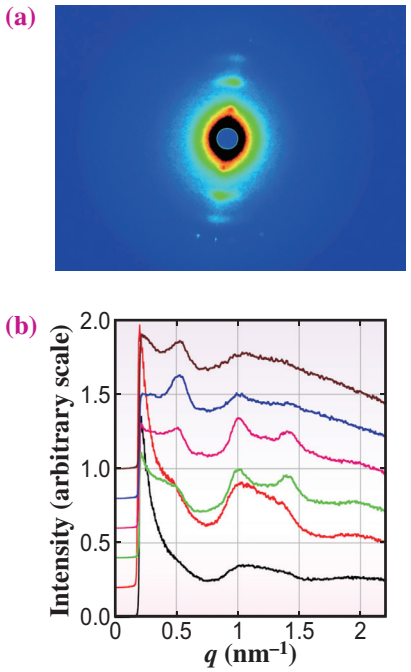

Fig. 2. **(a)** X-ray diffraction from human skin. Skin surface is horizontal. Diffraction spots arise from cholesterol crystals in skin. **(b)** Diffraction profiles at different depths. The sample was raised with 5 μm steps from the black to the brown curve so that the black curve is closest to the skin surface. The profiles are vertically displaced for clarity.

there is a broad peak at around $q = 2$ nm^{-1} which may be the 4th order of this periodicity. Figure 2(b) clearly shows that the peak at $q = 1.4$ nm^{-1}, which was observed as a shoulder in a previous report on human skin [3], is indeed a separate peak. However, it cannot be indexed on the order of the peak at 1.03 nm^{-1}. The intensity ratios and widths of these peaks vary at different depths of skin.

Based on these experimental results, we attempted to explain them in terms of lipid structure models. To account for the observed diffraction profiles, models of electron density distribution were constructed. These are based on the electron micrographs of Swartzendruber *et al.* [4] but the distance between bands and their heights did not strictly follow the micrographs which were obtained from the sections of chemically fixed, dehydrated, and stained samples. The simplest unit in the model consists of a central density minimum and two minima that were at 5.0 nm

on either side of the center with the same depth (Fig. 3(a)). Profiles of all minima were assumed to be Gaussians. Diffraction intensity expected from this unit gives broad peaks at around $q = 0.55$ and 1.1 nm^{-1} (Fig. 3(d)). When two such units were superposed with a separation of 13.0 nm (Fig. 3(b)), the resultant electron density profile resembles a model of tri-lamellar structure (the so-called Landmann unit). X-ray diffraction expected from this structure has peaks at $q = 0.5$, 1.0 and 1.3 nm^{-1} (Fig. 3(d)). With three units (Fig. 3(c)), there are small additional peaks other than the major three peaks (Fig. 3(d)). When the diffraction intensities from the one-, two- and three-unit models were added with a ratio of 3:3:1, the resultant profile (Fig. 3(e)) resembles the observed profile with peaks at $q = 0.56$, 1.04, 1.41 nm^{-1} and a broad baseline between the latter two peaks. This was found to be the best combination to match the experimental profile.

Based on the discussion by Swartzendruber *et al.* [4], the unit in our model represents the corneocyte lipid envelopes and their shared monolayer, while the two-unit model is made of two closely apposed bilayers. It has been shown by electron microscopy that intercellular spaces are mostly filled by either one, two, or three such units. It was indeed found in this study that the X-ray diffraction pattern from SC can be explained by summation of diffraction patterns from different numbers of units. A previous temperature-dependence study on porcine SC suggested that the peaks at $q = 1.0$ and 1.4 nm^{-1} originate from different lipid phases [5]. However, the present simulation demonstrates the possibility that they may arise from a single phase of lipids arranged with two and three repeats of the basic unit. This new interpretation may be used as a basic assumption in interpretation of X-ray diffraction from intact SC.

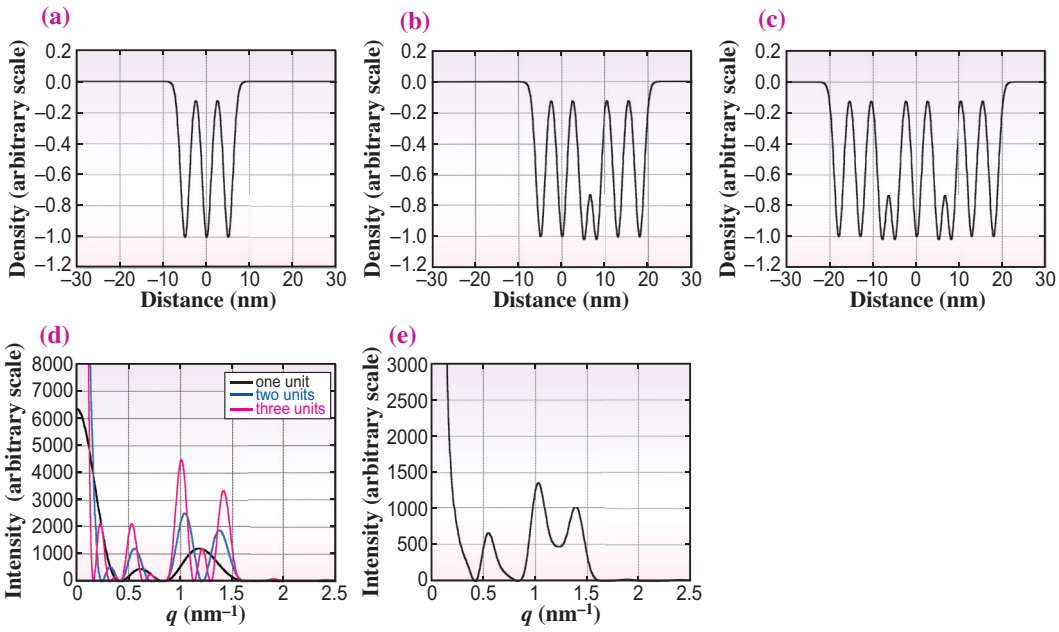

Fig. 3. Electron density models of lipids in SC and calculated diffraction from them. **(a)** An electron density model of one lamellar unit. There is an electron sparse region, corresponding hydrocarbon chains of lipids, in the center and there are two such regions at 5 nm on each side. All low electron density regions are simulated by a Gaussian with a full-width at half maximum of 3.5 nm. **(b)** A model in which the model (a) was duplicated in the right with a shift of 13 nm. **(c)** A model with three identical units. The third unit is shifted by –13 nm from the first unit. **(d)** X-ray diffraction expected from the one-unit model (black), two-unit model (blue) and three-unit model (red). **(e)** Mixture of one-, two-, and three-unit models with a ratio of 3:3:1.

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References

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