Industrial Applications

X-Ray Diffraction Analysis of Intercellular Lipid Structure in Human Stratum Corneum Collected by Less Invasive Method

Stratum corneum (SC), the outermost layer of skin, is an important organization as a barrier function of a mammalian skin, which regulates not only the evaporation of water from the body but also the invasion of exogenous hazards. SC is a very thin layer of about 10 µm and composed of corneocytes and an intercellular lipid matrix [1]. The ordered structure of the intercellular lipid matrix plays an important role in skin barrier function. Although some skincare cosmetics claim their efficacy on the barrier function, precise mechanisms on an intercellular lipid structure remain to be elucidated. Recently, some structural analyses of intercellular lipids in mammalian SC by X-ray diffraction have shown more detailed lipid structure models [2,3]. In mammalian SC, the longitudinal arrangement of lipid molecules, consisting of long and short lamellar structures with repeat distances of about 13 nm and 6 nm, respectively, has been observed by small-angle X-ray diffraction (SAXD). In the lateral arrangement of lipid molecules, hexagonal and orthorhombic hydrocarbon-chain packing has been observed by wide-angle X-ray diffraction (WAXD). However, these previous studies were often carried out using nonhuman SC samples, and studies with human SC have been limited. The purpose of this study is to establish a technique for analyzing the structure of intercellular lipids using synchrotron X-rays with a very small amount of SC sample collected less invasively from a healthy human volunteer.

SC samples were collected from the forearm and cheek of a healthy volunteer with a water soluble adhesive tape. SC samples were isolated by extensive washing with water to remove adhesive materials. After adjusting the water content of the isolated SC samples to about 30 wt%, a piece of each sample (about 3 mg) was immediately sealed in a capillary tube before X-ray diffraction measurements.

X-ray diffraction measurements were performed at beamline BL40B2 using 15 keV X-rays, and the scattered X-rays were detected using an imaging plate system (R-AXIS IV; Rigaku, Tokyo, Japan). The sample-to-detector distance was about 400 mm and the exposure time was 30 s. The diffraction pattern was circular-averaged to obtain a radial intensity profile.

The temperature of the sample was controlled using a temperature regulator (FP900 Thermo System; Mettler-Toledo K. K., Tokyo, Japan) and measured with a thermocouple embedded in the sample holder. All the experiments were performed in a heating scan from 15 °C to 120 °C at a rate of 0.5 °C min⁻¹. The X-ray diffraction profile was recorded every 2.5 °C.

The profiles of the human SC sample at 25 °C, 45 °C, and 85 °C in the small-angle and wide-angle regions are shown in Figs. 1(a) and 1(b), respectively. The value of \( S \) is given by \( S = \frac{2}{\lambda \sin \theta} \), where \( \lambda \) is the wavelength of the X-ray and \( 2\theta \) is the scattering angle.

![Fig. 1. X-ray diffraction profiles of human SC. (a) Small-angle and (b) wide-angle regions at 25 °C, 45 °C and 85 °C. Each plot is moved upward successively so as to see it easily. The reciprocal spacing is expressed by \( S = \frac{2}{\lambda \sin \theta} \), where \( \lambda \) is the wavelength of the X-ray and \( 2\theta \) is the scattering angle.](image-url)
Among others (Fig. 1(a)). At 25 °C, two diffraction peaks (S=2.4 nm⁻¹ and 2.7 nm⁻¹, corresponding to spacings of 0.42 nm and 0.37 nm, respectively) were observed superposed on a broad hump peak derived from soft keratin (Fig. 1(b)). Therefore, we could obtain a sufficient number of diffraction patterns for the analysis of a small amount of human SC sample.

The SAXD and WAXD data from the SC are plotted against temperature in Figs. 2(a) and 2(b), respectively. The intensity of the X-ray profile is represented by a contour line. On SAXD (Figs. 1(a) and 2(a)), as the temperature increased, the intensity for the lamellar structure with S=0.0736 nm⁻¹ decreased and that for the lamellar structure with S=0.16 nm⁻¹ also decreased. The latter peak deviated to a higher angle and disappeared above 80 °C. On WAXD (Figs. 1(b) and 2(b)), around 40 °C, the SC lipids underwent a structural transition from the orthorhombic state to the hexagonal state, which was consistent with the previous study on mouse SC by Hatta et al. [4]. The diffraction peaks at S=2.4 nm⁻¹ and 2.7 nm⁻¹, which were derived from the hydrocarbon-chain packing of the orthorhombic state, disappeared at about 40 °C. Above 40 °C, the diffraction peak at S=2.43 nm⁻¹, which was due to the hydrocarbon-chain packing of the high-temperature hexagonal state, appeared instead. Above 40 °C, the lattice constant of the hydrocarbon-chain packing in the high-temperature hexagonal state gradually increased with an increase in temperature. Above 80 °C, the high-temperature hexagonal state, as well as the above-mentioned lamellar structure in the SAXD, disappears. These phase transitions were reversible, since recrystallization was observed upon cooling from 120 °C to 25 °C (data not shown).

It is interesting that a distinct phase transition occurs around the body temperature, which may explain the result obtained in the previous study indicating that percutaneous absorption of some drugs depends on the temperature [5]. We consider that the X-ray diffraction analysis of intercellular lipid structure in human SC obtained by tape stripping is a promising technique for the development of new cosmetics based on the structural change of intercellular lipids. At present, a further detailed examination about the relationship between the intercellular lipid structure in human SC and the barrier function of skin is necessary.

Fig. 2. Intensity contour maps of SAXD (a) and WAXD (b) in stratum corneum of human. The intensities are shown by colored lines. High-to-low intensities are shown in red-to-violet contours.

References