Release of Ceramide Molecules from Ceramide-Containing UV-curable Acrylic Adhesive Gel Sheet Affixed to Human Skin

Hiroshi Takahashi¹*, Ryota Watanabe², Kenichi Nishimura², and Taro Moriwaki³

¹ Division of Pure and Applied Science, Faculty of Science and Technology, Gunma University, 4-2 Aramaki, Maebashi, Gunma 371-8510, JAPAN
² Medical Engineering Laboratory, ALCARE Co., Ltd., 1-21-10 Kyojima, Sumida-ku Tokyo 130-0046, JAPAN
³ Japan Synchrotron Radiation Research Institute (JASRI/Spring-8), 1-1-1 Kouto, Sayo, Hyogo 679-5198, JAPAN

Abstract: Ceramide, an intercellular lipid of the stratum corneum, plays an essential role in making the skin barrier. One problem with the use of medical adhesive tape or sheets for skin is that their repeated attachment and detachment may cause some damage to the skin. An attempt has been made to eliminate this problem by mixing ceramide into the adhesive of sheets, and has delivered excellent clinical results. This study aimed to investigate whether ceramide is transferred from the adhesive with added ceramide to the skin. An adhesive sheet was prepared by adding synthetic ceramide (CER) to UV-curable acrylic adhesive gel. After affixing the adhesive sheet to human skin for a certain period, it was peeled off and cut perpendicular to the adhesive surface. Synchrotron micro-infrared spectroscopy of the sectioned samples showed that the ceramide concentration in the gel sheet decreases as the application time to human skin increases. This is thought to be due to the release of CER from the gel sheet.

Key words: ceramide, acrylic adhesive gel, human skin, skin protectant, micro-infrared spectroscopy

1 Introduction

Skin functions as a barrier preventing the entry of microorganisms and pathogens into the human body and protects the body from various external stimuli such as chemical and physical stress ¹. Ceramide, an intercellular lipid, is present in the stratum corneum, that is the outermost layer of the skin. The presence of ceramide has been widely recognized to be particularly important for skin barrier functions ², ³. It is well known that the skin of atopic dermatitis patients is deficient in ceramides ⁴, ⁵. It has also been reported that creams containing ceramide improve the barrier function of the stratum corneum of atopic dermatitis patients ⁶, ⁷.

Medical adhesive tape or sheet is applied directly to patients’ skin to affix medical instruments or protect wounds. Some medical devices are also attached directly to the skin using adhesive materials. When these tapes or sheets are peeled off, the skin epidermis layer may be removed or damaged. In a patient who has an ostomy, the adhesive needs to be repeatedly attached to and peeled from the skin over a long period to attach and remove the stoma device. This repeated use may induce dermatitis ⁸, ⁹. It is a serious problem for older people with weak skin function ¹⁰.

To address this problem, the use of several skin protection agents has been tried; however, these skin protection agents were not completely effective, i.e., skin disorders still occurred in some cases. An attempt ¹¹ was made using ceramide, one of the key skin lipids providing the barrier function of the stratum corneum as described above. In this attempt, ceramide was added to the adhesive used on the surface of the stoma device. The ceramide-containing adhesive gel sheets were applied on healthy subjects and ostomates, and the transepidermal water loss (TEWL) in the subject’s skin was measured. Then the degree of skin damage was evaluated from the TEWL value. The results showed that not only did the addition of ceramide contribute to maintaining the skin barrier function, but also a slight improvement of the skin barrier function was observed ¹².

In our previous study ¹², we initially examined the assembly structure of ceramide molecules in an adhesive gel material to obtain fundamental insight into the mechanism...
of the skin protecting effect by adding ceramides to the gel. The investigation was performed using acrylic photocuring adhesive gel\textsuperscript{13, 14} as an adhesive material. It was found that ceramide in adhesive gel forms a bilayer structure similar to that in the actual skin stratum corneum, depending on the conditions at the time of production\textsuperscript{12}. It was also found that lamellar structures are formed and the hydrocarbon chains of ceramide are packed in an orthorhombic two-dimensional lattice\textsuperscript{13, 14}.

From the above-obtained result, it can be theorized that the ceramide-containing gel sheet itself acts as a kind of alternative skin, resulting in the maintenance of the barrier function of the skin. Another possibility is that the ceramide is transferred from the ceramide-containing adhesive to the skin. The ceramide transferred would compensate for the decrease in ceramide in the stratum corneum that is induced by repeated attachment and peeling of the sheet. The transfer of ceramide may also contribute to the recovery process. Next, we investigated a sectioned sample after pasting the ceramide-containing adhesive sheet on cultured skins in order to examine the transfer of ceramide molecules from the adhesive sheet to the skin\textsuperscript{15}. The analysis of the data obtained using synchrotron micro-Fourier-transform infrared (micro-FTIR) spectroscopy indicated that a significant decrease in the amount of ceramide in the adhesive sheets occurred near the interface between the sheet and the cultured skin.

Cultured skin is not exactly the same as human skin\textsuperscript{16}. For example, it has been reported that the lipid composition ratio of cultured skins differs slightly from that of human skin\textsuperscript{17}. The present study aimed to re-confirm the results obtained for cultured skins by performing the same measurements using real human skin. The results obtained in this study not only support the previous results indicating the release of ceramide molecules from the gel sheet but also give us new insights. Compared with the results from cultured skin, the release of ceramide molecules from a deeper region of the gel sheet was observed for a gel sheet affixed to human skin. The slight difference in the experimental results obtained between cultured skin and real human skin seems to be due to the fact that the cultured skin never moves but human moves during attachment of the gel sheet.

2 Experimental Procedures
2.1 Materials and sample preparation

The adhesive material and synthetic ceramide used in this study were identical to those used in our previous studies\textsuperscript{13, 15}. The complete chemical name of the synthetic ceramide (CER) is (2S,3R)-2-octadecanoylamino-octadecane-1,3-diol, which was obtained from Takasago International Corp. (Tokyo, Japan). According to the supplier’s information, both the chemical and optical purity of the CER are more than 95%. The synthetic method for CER has been reported by Ishida \textit{et al.}\textsuperscript{18} CER is composed of a dihydrophosphoglycerine backbone and a single non-hydroxy saturated fatty acid chain and has the same stereochemical configuration as that of a naturally occurring ceramide\textsuperscript{19}. According to the International Nomenclature of Cosmetic Ingredients, CER is classified as ceramide NG. This type of ceramide has been reported to be found in the skin tissues of various body parts\textsuperscript{20}. The fatty acid chain of CER has 18 carbon atoms. The phase behavior of CER has been reported by Takahashi \textit{et al.}\textsuperscript{20} Prepolymers with a mean molecular weight of \(~200,000\) of a UV-curable acrylic adhesive resin material were purchased from BASF (Ludwigshafen am Rhein, Germany). The trade name is acResin\textsuperscript{8}, and it is made of benzophenone groups and the polymer of butyl acrylate\textsuperscript{13, 14}.

The method for preparing adhesive sheet samples was similar to that reported in previous papers\textsuperscript{12, 15}. Only a brief description is given here. A mixture of CER powder and acResin\textsuperscript{8} prepolymer was heated in order to completely melt the CER. The concentration of CER was 9% by weight. This heating process was needed in order to have uniform mixing. The hot-mixed sample was then spread on a hot plate at 125°C to create a sheet with a thickness of \(~200\) \(\mu\)m. During irradiation of UV light to cure the sample, the sheet sample was kept at 80°C. Sample sheets were formed by laminating four sheets, each having a thickness of \(~180\) \(\mu\)m. Both sides of the adhesive gel sheet were covered by a 50-\(\mu\)m PET film (PET50 x1 A3, Nippa Corp. Osaka, Japan) to facilitate handling. When the sheet was affixed to the skin, the PET films were removed. The surface opposite to the adhesive surface was then covered with a urethane dressing film (Multi Fix-Roll, Alcare Co., Ltd. Tokyo, Japan).

The sample gel sheets were affixed to the abdominal skin of a healthy volunteer (male, 20s) for specified periods (10 min, 1 day, 3 days, 1 week) (Fig. 1) and then peeled off. According to the method described elsewhere\textsuperscript{15}, the sectioned samples were prepared using a microtome. A longitudinal section of the sample was set to a cutting thickness of 10 \(\mu\)m. The section was attached to a support on a CaF\textsubscript{2} plate. When the actual thickness of the sample was observed with a three-dimensional laser microscope (Lasertec OPTELICS H 1200, Yokohama, Japan), the thickness of the sectioned sample was not uniform and varied from \(~5\) \(\mu\)m to \(~90\) \(\mu\)m depending on the position (Figs. 2 and 3). This variation is probably due to the fact that the sample preparation is extremely difficult because of the considerable softness of the adhesive gel sheets.

2.2 Measurements

Micro-FTIR spectroscopic measurements using synchrotron radiation were performed at the SPring-8 infrared mi-
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Fig. 1 Photograph of CER-containing UV-curable acrylic adhesive gel sheet affixed to the abdominal skin of a healthy volunteer.

Fig. 2 Optical microscopic image of sectioned sample of the CER-containing UV-curable acrylic adhesive gel sheet attached to human skin for 3 days. The bottom side corresponds to the skin-attached side. The surface of the skin-attached side was rough due to peeling from the skin. The scale bar is 400 \( \mu m \).

Fig. 3 Plots of the estimated thickness of sectioned samples of CER-containing adhesive gel sheet affixed to the skin by a three-dimensional laser microscope as a function of position. The attached time of the skin were (a) 10 min, (b) 1 day, (c) 3 days, and (d) 1 week, respectively. Each increment of the vertical axis corresponds to 100 \( \mu m \). In order to easily be recognized, the zero height position of each sample was moved. The position of zero corresponds to the edge of the skin-attached side of each sheet. The data of (c) had been already presented in our previous paper\(^{15}\).

circular plate (Pier Optics Co., Ltd, Tatebayashi, Japan) was used as an IR measurement substrate. The diameter and thickness of the CaF\(_2\) plate were 18.0 mm and 1.0 mm, respectively.

X-ray diffraction measurements were performed at the Photon factory small-angle X-ray scattering BL10C station\(^{23}\). The measurement conditions such as camera lengths and a detector were identical to those described in the previous study\(^{22}\).

Measurements of the thickness of the sliced sample were carried out using a Lasertec OPTELICS H 1200 three-dimensional (3D) laser microscope (Lasertec Corporation). The observation procedure was identical to that of the previous study\(^{15}\).
### 3 Results

#### 3.1 X-ray diffraction of adhesive gel sheet containing ceramide

As a first step, we investigated the micro-structure of the sample and the stratum corneum of human skin using X-ray diffraction. Figure 4 shows X-ray diffraction patterns of the CER-containing UV-curable acrylic gel sheet (10 wt% CER concentration) and stratum corneum of human skin obtained from a healthy male heel. The patterns were recorded at room temperature (≈22–24°C). The adhesive gel sheet sample (10 wt% CER) investigated here was not exactly identical to those (9 wt% CER) measured by micro-FTIR experiments.

For the CER-containing gel sheet sample, lamellar diffraction peaks are observed clearly up to the third order, and the lamellar spacing is 4.08 nm. In the wide-angle region, two peaks with spacings of 0.45 nm and 0.38 nm are observed, indicating that the hydrocarbon chains of CER are packed into an orthorhombic two-dimensional lattice. These structural parameters agree with those of samples maintained at 60°C for 6 days reported in our previous paper. The broad bands observed at approximately ~5 nm⁻¹ and ~14 nm⁻¹ (Fig. 4(a)) can be assigned to the matrix gel sheet because both bands were also observed for a UV-curable gel sheet sample without CER added. The sharpness of the wide-angle diffraction peaks (Fig. 4(a)) was slightly broader than that of the previous ones. This is probably because the thermal histories of the samples investigated here and previously are somewhat different from each other.

As a reference, the diffraction pattern of the stratum corneum of human skin is also shown in Fig. 4(b). In the wide-angle region, there is a fairly weak peak with spacing of 0.41 nm (S = 15.3 nm⁻¹), as indicated by an arrow in Fig. 4(b). It has been reported that two lamellar structures with different lamellar spacings are observed for various animal skins, including human skin. One is a long-spacing lamellar structure and the other is a short-spacing lamellar structure. The lamellar periodicity of the former is ~13 nm, and the periodicity of the latter is ~5–8 nm. It has been proposed that the long-spacing lamellar structure is composed of long-chain ceramide molecules, such as ceramide(EOS). The long chain of ceramide(EOS) is composed of a linoleic acid linked to an ω-hydroxy fatty acid (EO) with a chain length of 30–32 carbon atoms. The short-spacing lamellar structure is thought to contain short and middle-chain ceramide molecules. The fatty acid chains of such a ceramide contains 16–24 carbon atoms. Because of the relatively short camera length used to observe a wide angular region, the resolution of the small angle region of the present X-ray diffraction data was insufficient to detect diffraction peaks originating from the long-spacing lamellar structure. Therefore, only the diffraction peak originating from the short-spacing lamellar structure could be observed in this study, as shown in Fig. 4(b). The lamellar spacing was 4.9 nm, slightly shorter than the reported value (5.8 nm) for the short-spacing lamellar structure of human stratum corneum. This may be due to the lower water content of the heel stratum corneum as compared with that of other parts of the body. For the short-spacing lamellar structure of the stratum corneum of a hairless mouse, it has been reported that the lamellar spacings change from 6.6 nm to 5.8 nm as the water content decreases from 50% w/w to 12% w/w.

The present X-ray diffraction data show that CER in the UV-curable acrylic gel sheet also forms a lamellar bilayer structure that is quite similar to that formed by pure CER. The relatively sharp diffraction peaks in the wide-angle region indicate that CER in the UV-curable acrylic gel sheet is not fluid but a solid-state at room temperature. The fact that the observed lamellar spacing of CER in the gel resin is shorter than that of heel skin is likely due to chemical composition differences. The former lamellar structure is composed of only a single kind of molecule, i.e., CER, but the latter lamellar structure is composed of not only various types of ceramide molecules but also different fatty acids, cholesterol-esters, etc. These results indicate that a lamellar structure similar to that of real human skin cannot be completely reproduced using only a single type of ceramide.
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3.2 Measurement of sectioned sample thickness by laser microscope

Figure 2 shows an example of an optical microscopic image of the sectioned gel sheet sample. The sheet had been attached to human skin for 3 days. Just before performing the micro-FTIR measurements, the actual thickness of the sample to be measured was observed with a three-dimensional laser microscope. The result showed that the thickness of the section was not uniform and varied from ~5 µm to ~90 µm depending on the position (Fig. 3). This variation is probably due to the fact that sample preparation is extremely difficult due to the considerable softness of adhesive gel sheets as pointed out previously.15

In addition to the thickness, the widths of the sections was also not uniform. Because the sample sheets were made from four sheets with ~180 µm thickness, the width of the sectioned samples should be ~720 µm. All observed values, however, were smaller than 720 µm. The widths were estimated from the profile shown in Fig. 3. The following results were obtained: 676, 537, 434, and 525 µm for the attached periods of 10 min, 1 day, 3 days, and 1 week, respectively, showing a trend that the longer the time the sample was applied to the skin, the shorter the width. This phenomenon was never observed on sheets containing adhesive gel sheets, which can result in compression of the sheets.

3.3 Micro-FTIR spectroscopic measurements

In the gel sheet samples studied here, only CER contains nitrogen atoms, except for the urethane dressing film. Data for the urethane film region were not recorded in this study. A nitrogen atom is contained in the amide bond of CER. The amide bond gives several characteristic peaks in the IR spectrum. In the measured wavenumber range of this study, peaks for amide I (1650 cm\(^{-1}\)) and amide II (1540 cm\(^{-1}\)) derived from the amide bond appear in the absorption spectrum (Fig. 5). The integrated intensity of these peaks is proportional to the amount of CER. Hence, if the intensity is obtained for each location, the amount of CER at each measurement location can be evaluated. However, since the thickness of the sectioned sample is not constant (Fig. 3), the intensity data themselves are not directly proportional to the CER concentration in the adhesive gel at the measurement position. Although we intended to prepare a sample with a thickness of 10 µm, there was considerable variation from point to point, as revealed from the thickness evaluation using a three-dimensional laser microscope (Fig. 3). In order to compensate for the thickness variation, we used the same method developed in our previous study15 for experiments using cultured skin. In this method, the peak intensity due to the C=O stretching vibration of adhesive ester observed at 1660–1830 cm\(^{-1}\) was used to estimate the amount of adhesive gel for each measurement position. By standard FTIR measurement, we confirmed that no ester peak appears in the sample of CER alone and no amide I or II peak appears in the case of the adhesive gel alone. Thereby, the intensity of the ester peak observed at 1660–1830 cm\(^{-1}\) was used to estimate the amount of adhesive gel material and to normalize the amide I data. The reliability of this estimate depends on the accuracy of the measured intensity of the IR peak. As tests of reliability of the C=O stretching vibration intensity, a comparison was performed between the height values estimated from laser microscopy and from IR data (Fig. 6). In this analysis, it was assumed that the integrated intensity of the C=O stretching vibration peak is proportional to the height of the sample at each measurement position. The two values are almost the same, but not exactly (Fig. 6). For the IR measurements, we attempted to measure at the same position as much as possible, judging from the sample shape captured by the microscope, but it is impossible to scan the exact same position. This is likely the reason for the small difference in Fig. 6. A similar degree of agreement was found for the other samples. Based on this overall degree of agreement, we judged the present IR measured values to be sufficiently reliable.

Figure 7 is a plot of the average value obtained by dividing the strength of amide I peak intensity by that of the ester (1660–1830 cm\(^{-1}\)) peak intensity against the distance.
from the skin adhesive surface. The error bars correspond to the standard deviation of the different data sets. For the data of 10 min and 3 days, error bars are not displayed because we could obtain only two data sets due to accidental trouble of data acquisition. The horizontal axis is the distance from the origin position \( u \) of the distance, i.e., the interface between the skin and the gel sheet was determined from the overall IR spectrum change. In Fig. 5 of our previous study using cultured skin samples, the vertical values were normalized ratios, i.e., the ratio of the initial state in which no CER molecules were released from the gel sample was normalized to be one. Here, we did not use such a normalization. Figure 8 shows the mean values of the data displayed in Fig. 7.

The ratio of amide I to ester peak intensities on the vertical axis in the figure is proportional to CER concentration at the measured positions. As can be seen from Figs. 7 and 8, the longer the application time to human skin, the lower the intensity ratio value, i.e., the CER concentration. This implies that CER was released from the adhesive gel sheet affixed to human skin.

### 4 Discussion

In our previous study of CER-containing gel sheets affixed to cultured skins, a decrease in CER concentration was observed only near the interface, i.e., at a distance of less than approximately 50 \( \mu m \). On the other hand, a decrease in CER concentration in gel sheets affixed to human skin occurred at greater distances. Let us discuss why the results differed between human skin and cultured skin.

First, consider the mechanism of how CER molecules are released from the gel sheets. We confirmed that when heated to above the melting point of CER, CER in the liquid state flows out from the gel sheet (data not shown). At a room temperature, however, the CER molecules are in a nearly crystal-like solid-state, as judged by the fact that relatively sharp X-ray diffraction peaks are observed in the wide-angle region (Fig. 4). The melting temperature of CER in the adhesive gel sheet (approximately 110°C) has
been confirmed to be almost the same as that of pure CER\textsuperscript{12}. The temperature of the sheet samples would be raised above room temperature when attached to the human body, but would not reach the melting point. In order for CER molecules to move in the adhesive gel matrix, CER molecules would need to be in a nearly fluid state. We have previously proposed a possible mechanism\textsuperscript{15}, as follows. Some oily substances from skin, such as sebum, may help CER to become a liquid by solubilizing CER. Some components of sebum are liquid that can therefore penetrate inside the gel sheet. Sebum is composed mainly of acylglycerols, free fatty acids, wax esters, squalene and sterols\textsuperscript{20}. The detailed chemical composition of human sebum obtained from the central forehead has been investigated by the use of high-performance liquid chromatography and electrospray mass spectrometry\textsuperscript{30}. It revealed that 16:0, 16:1, and 18:1 hydrocarbon chains are predominant in the lipids of sebum. Lipids with 16:1 and 18:1 chains are expected to be in the liquid state at average body temperature. Generally, the solubility of ceramides in oils is not high. A study of emulsion systems containing ceramides has shown that the presence of charged additives increases the solubility of ceramides in oil\textsuperscript{31}. Some components of sebum might contribute to an increase in the solubility of the CER. The amount of oily substances released from living human skin is likely to be greater than that from cultured skin.

In addition, various physical stresses (compression, tension, torsion, etc.) induced by activities of the human body in daily life could also contribute to solubilization of CER in the adhesive gel matrix. Physical stresses actually compress the gel sheets (Fig. 3). These physical stresses would be expected to have the same effect as a vibrator, promoting the mixing of oily substances and CER molecules. For cultured skins, owing to no such effects, oily substances cannot penetrate deep inside the gel sheet; therefore, it is inferred that the decrease of CER concentrations occurs only in the region very close to the interface.

5 Conclusion

In conclusion, the present experiment showed a decrease in the CER concentration of CER-containing adhesive gel sheets as the time applied human skin increased. This can be interpreted as indicating that the ceramide has moved to the human skin. However, ceramide molecules originally present in human skin cannot be distinguished from transferred CER by the method used here. Hence, the amount of transferred CER in skin could not be directly estimated. This could be a future issue to explore. Future studies should also investigate whether the CERs released from the adhesive gel sheet remain only on the skin surface or penetrate deeper into the stratum corneum.

A comparison of this study with our previous study\textsuperscript{15} showed that there is a difference in the behavior of CER concentration depending on whether cultured skin or living human skin is used. This difference was confirmed by analyzing the data with micron-order position resolution obtained at different positions. In order to collect such high-quality data, micro FTIR measurements using powerful and small beam size synchrotron radiation were essential. The results of this study remind us that living human skin patching experiments must be performed to obtain data that reflects the effects of actual human behavior. This is of significant importance. On the other hand, data fluctuation due to differences in human activity never occurs in experiments using cultured skins, which can be advantage for some purposes.

The reported measurements were performed on only one human subject. As described in the discussion section, the result should depend on the daily activities of subject. By conducting experiments on a large number of subjects, it should be examined whether differences in daily activity have a significant effect on the results. That is, an activity-controlled experiment should be conducted to explore if the results correlate with activity. This is also an important next task.

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