The characteristics of biological materials are closely related to their water content. Consequently, measurements of the dynamics of water molecules are important in order to understand the structures of hydrous materials and their biological functions. Human skin is composed of three layers termed, sequentially inward from the surface, the epidermis, dermis, and subcutaneous tissue. The water content of the epidermis is lower than that of the dermis, and it behaves as a protective barrier against drying and external irritation. The existence of bound water is often suggested, even for human skin. It is not easy to determine the variation of the water content with depth, and measurements are difficult, since water molecules exhibit complex variations on different temporal and spatial scales.

Dielectric measurements using time-domain reflectometry (TDR) make it possible to observe relaxation processes that reflect
the molecular motions of water. The relaxation process is caused by the formation and disappearance of dynamic clusters of water molecules. For free water molecules, the relaxation time is \( \tau = 8.27 \) ps at 25\(^\circ\)C.\(^{10-13}\) This relaxation time is generally smaller than that of biological macromolecules.

The water content of human skin has been studied extensively by various methods.\(^{14-16}\) Pratchyapruit et al\(^{17}\) suggest that the skin of the eyelid remains soft by maintaining sufficient hydration on the skin surface. This example demonstrates that water in the skin is related to biological function.

In our previous studies, we obtained information about the dielectric properties in the stratum corneum of human skin.\(^{18,19}\) We also have reported that the healing of human skin after a burn is correlated with the time dependence of its free water content.\(^{20}\) Applying dielectric measurements of skin to the area of sports medical science, we were able to observe the large-scale traversal of water molecules into the body during exercise.\(^{21}\) Dielectric spectroscopy can be employed for non-invasive and non-destructive measurements, including in vivo measurements of human skin. These reports clearly indicate that skin measurements using dielectric spectroscopy can provide an important method for characterizing the state of the skin through observations of the molecular behavior of water.

Recently, measurements of the moisture content of skin have been performed using confocal Raman spectroscopy.\(^{22}\) This experimental technique makes it possible to observe the moisture content at a shallow depth in areas of the skin such as the stratum corneum and epidermis. On the other hand, dielectric spectroscopy yields a value averaged over the penetration depth of the electric field up to the skin surface contacted by the electrode. Furthermore, by increasing the electrode diameter it is possible to obtain information in deeper areas than can be studied with confocal Raman spectroscopy. We have previously reported a comparison of results obtained from these two measuring techniques.\(^{23}\) Observations of the dynamics of water molecules using dielectric spectroscopy are relevant not only to the water content but also to phenomena such as biological functions and characteristics in vivo. Therefore, dielectric spectroscopy is appropriate for in vivo measurements.

In dielectric spectroscopy of the skin, it is important to know which and how dielectric information in depth is obtained for substances with different water contents. Accordingly, we have examined the characteristic electric-field penetration depths of a two-layer dielectric model using water and a Teflon block.\(^{18,24,25}\) The penetration depth obtained from such measurements depends on the outer diameter of the coaxial electrode. However, the electrode diameter was only one of the unknowns in previous research. In this study, we have used TDR with open-ended coaxial electrodes having different electric-field penetration depths to perform dielectric measurements of an acetone-Teflon double layer and of human skin from various parts of the body, in order to study the distribution of water content in the human skin.

2 | MATERIALS AND METHODS

2.1 | Acetone-Teflon double-layer system for the characterization of coaxial electrodes

A block diagram of the apparatus employed for the characterization of coaxial electrodes is shown in Figure 1. We purchased a flat, 10-mm-thick Teflon sheet with static permittivity \( \varepsilon_r = 2.1 \) from FLON INDUSTRY. We placed a glass bottle on a jack and filled the bottle with acetone (with static permittivity \( \varepsilon_a = 21 \) at 20\(^\circ\)C\(^{26}\)). We then placed a Teflon sample in the bottom of the bottle to form an acetone-Teflon double-layer system. We first placed the end of the electrode into contact with the surface of the Teflon, and we then changed the vertical distance between the Teflon surface and the end of the electrode gradually using the jack while we performed the dielectric measurements. We determined the distance using a digital indicator. We used 50 \( \Omega \), semi-rigid coaxial cables (SUHNER) with outer diameters of 0.86, 1.19, 2.20, 3.56, and 6.35 mm as the electrodes for the TDR measurements at room temperature (20.0 ± 0.5\(^\circ\)C).

2.2 | Human skin measurements on various parts of a body

We measured various parts of the bodies of two examinees (A: male, age 24; B: female, age 26) using TDR, with the same electrodes as we used for the acetone-Teflon double-layer measurements at room temperature, 20.0 ± 0.5\(^\circ\)C, with humidity 50 ± 1%. The body parts we measured were the cheek, the palm side of the forearm, the palm, and the sole of the foot. We also used TDR to measure the same body parts of two other examinees (C: male, age 24; D: female, age 22) with an electrode having an outer diameter of 2.2 mm. We
washed the measured body parts with soap and wiped them with Kimwipe before the measurements. In order to restore the state of the skin prior to the measurements, we asked each examinee to remain still in the examining room for 30 minutes so as not to sweat.

2.3 Time-domain reflectometry measurements

We employed a digitizing oscilloscope mainframe (HP 54120B) with a four-channel test set (HP 54124A) as the TDR system.\textsuperscript{27-31} This enabled us to make dielectric measurements—especially for the relaxation process due to the molecular dynamics of free water—over the frequency range from 100 MHz to 30 GHz. We observed the reflected microwave signals, which include information about the dielectric properties of the sample in response to the incident step pulse.

The complex permittivity of the sample can be expressed by the basic equation of the TDR method:\textsuperscript{27-29}

\[
\varepsilon' = \frac{c'}{v_0} - \frac{r}{v_0 + r} X \cot X \tag{1}
\]

where \(c'\) is the speed of propagation in the coaxial line, \(v_0\) and \(r\) are the Laplace transforms of the incident and reflected pulse waveforms, \(X \cot X\) takes into account propagation and multiple reflections in the sample and the coaxial line geometry, and \(\gamma d\) is a correction coefficient. There is reference method\textsuperscript{27-29} using the difference between the reflected wave of a standard sample having a known complex dielectric constant and the reflected wave of an unknown sample, which was used because it is more accurate.\textsuperscript{27-29} The values of the correction coefficients \(\gamma d\) are listed for each electrode in Table 1. We also calibrated the open-ended probes using standard samples (air, acetone, and water) with known dielectric properties.

We performed the TDR measurements using a contact-type coaxial electrode with an open-ended tip in direct contact with the object to be measured. The TDR measurement requires an electric field generated around the tip of the electrode. The electric lines of force reach from the central to the outer conductors through the interior of the object. The dielectric information is obtained in the region where a relatively large electric field is applied around the electrode tip. Therefore, the electric lines of force necessarily depend on the diameter of the electrode. In this report, we use measurements of the dielectric information averaged over the penetration depth of the electric field as the basis for discussing the layered structure of the human skin.

3 RESULTS

3.1 Acetone-Teflon double-layer system

Figure 2 shows the relationship between the static dielectric constant \(\varepsilon_s\) and the distance \(l\) between the electrode and the Teflon surface for electrodes with outer diameters of 0.86, 1.19, 2.20, 3.56, and 6.35 mm. The \(\varepsilon_s\) values increase with increasing \(l\), and they asymptotically approach the value for acetone. The dielectric constant thus obtained is larger than \(\varepsilon_s = 2.1\), even when the end of electrode just touches the Teflon surface (\(l = 0\)), because there remains a small gap filled with acetone. The variation of the dielectric constant with position thus obtained shows exponential-like decays.

![Figure 2](image_url) Relationship of the measured dielectric constant to the distance between the Teflon surface and the electrode, for outer electrode diameters of 0.86, 1.19, 2.20, 3.58, and 6.35 mm

\begin{table}[h]
\centering
\caption{Values of correction coefficients}
\begin{tabular}{|c|c|c|c|c|}
\hline
\(\phi\) [mm] & 0.86 & 1.19 & 2.20 & 3.56 & 6.35
\hline
\(\gamma d\) [mm] & 0.059 & 0.082 & 0.15 & 0.29 & 0.46
\hline
\end{tabular}
\end{table}

\begin{table}[h]
\centering
\caption{Parameters for an electrode with an outer diameter of 0.86 mm}
\begin{tabular}{|c|c|c|}
\hline
\(l\) [mm] & \(\varepsilon_s\) & \(\ln (\varepsilon_s - \varepsilon_t)\)
\hline
0.00 & 5.43 & 2.75
\hline
0.01 & 11.04 & 2.30
\hline
0.02 & 13.91 & 1.96
\hline
0.03 & 16.13 & 1.58
\hline
0.04 & 15.91 & 1.63
\hline
0.05 & 16.53 & 1.50
\hline
0.06 & 16.40 & 1.53
\hline
0.08 & 18.83 & 0.78
\hline
0.10 & 18.56 & 0.89
\hline
0.20 & 20.70 & –1.21
\hline
0.30 & 20.97 & –3.44
\hline
\end{tabular}
\end{table}

\begin{table}[h]
\centering
\caption{Parameters for an electrode with an outer diameter of 1.19 mm}
\begin{tabular}{|c|c|c|}
\hline
\(l\) [mm] & \(\varepsilon_s\) & \(\ln (\varepsilon_s - \varepsilon_t)\)
\hline
0.00 & 5.11 & 2.77
\hline
0.01 & 6.82 & 2.65
\hline
0.02 & 7.81 & 2.58
\hline
0.03 & 8.58 & 2.52
\hline
0.04 & 8.81 & 2.50
\hline
0.05 & 9.66 & 2.43
\hline
0.10 & 13.34 & 2.04
\hline
0.15 & 15.33 & 1.74
\hline
0.20 & 18.91 & 0.74
\hline
0.25 & 19.07 & 0.66
\hline
\end{tabular}
\end{table}
3.2 | Human skin measurements

We used electrodes with various outer diameters to measure various parts of the body, and we observed the respective dielectric relaxation curves. We found the dielectric constant to be small when the electrode diameter was small. In addition, we obtained smaller values of the dielectric constant for body parts with larger skin thicknesses.

4 | DISCUSSION

4.1 | Characterization of the penetration depth of the electric field

The distance dependence shown in Figure 2 can be expressed approximately by the following equation: \[ \varepsilon_s = \varepsilon_t - \left( \varepsilon_a - \varepsilon_t \left[ 1 - \exp \left( \frac{-l}{l_0} \right) \right] \right), \] (2)

where \( \varepsilon_s \) is the dielectric constant of acetone, and \( l_0 \) is defined as the penetration depth of the electric field. The penetration depth has a characteristic value for each electrode, which is determined from Equation 2. The penetration depth is an average value, and some parts of the electric field lines reach deeper regions.

The dielectric constant \( \varepsilon_s \) and the distance \( l \) thus obtained are listed in Tables 4-6. As noted in Section 3.1 above, when the end of the electrode just touches the Teflon surface (\( l = 0 \)), the dielectric constant obtained from the measurements is larger than \( \varepsilon_t = 2.1 \) because of acetone in the gap between the end of the electrode and the Teflon surface. In our previous research, we pointed out that

| TABLE 4 | Parameters for an electrode with an outer diameter of 2.20 mm |
|----------|------------------|------------------|
| \( l \) [mm] | \( \varepsilon_s \) | \( \ln (\varepsilon_s - \varepsilon_t) \) |
| 0.00 | 6.62 | 2.67 |
| 0.02 | 9.89 | 2.41 |
| 0.04 | 13.41 | 2.03 |
| 0.06 | 15.36 | 1.73 |
| 0.08 | 16.88 | 1.42 |
| 0.10 | 17.00 | 1.39 |
| 0.15 | 17.63 | 1.21 |
| 0.20 | 18.53 | 0.90 |
| 0.25 | 18.93 | 0.73 |
| 0.30 | 19.57 | 0.36 |
| 0.35 | 19.83 | 0.16 |
| 0.40 | 20.12 | -0.13 |
| 0.45 | 20.22 | -0.25 |
| 0.50 | 20.60 | -0.92 |
| 0.60 | 21.19 | - |
| 0.70 | 20.95 | -3.00 |
| 0.80 | 21.19 | - |
| 0.90 | 21.07 | - |

| TABLE 5 | Parameters for an electrode with an outer diameter of 3.58 mm |
|----------|------------------|------------------|
| \( l \) [mm] | \( \varepsilon_s \) | \( \ln (\varepsilon_s - \varepsilon_t) \) |
| 0.00 | 5.27 | 2.76 |
| 0.02 | 10.11 | 2.39 |
| 0.04 | 10.89 | 2.31 |
| 0.06 | 11.22 | 2.28 |
| 0.08 | 11.49 | 2.25 |
| 0.10 | 11.88 | 2.21 |
| 0.15 | 13.50 | 2.01 |
| 0.20 | 14.74 | 1.83 |
| 0.25 | 15.12 | 1.77 |
| 0.30 | 15.90 | 1.63 |
| 0.35 | 16.89 | 1.41 |
| 0.40 | 17.55 | 1.24 |
| 0.45 | 17.98 | 1.11 |
| 0.50 | 18.1 | 1.06 |
| 0.60 | 19.15 | 0.62 |
| 0.70 | 19.51 | 0.40 |
| 0.80 | 19.7 | 0.26 |
| 0.90 | 20.24 | -0.27 |
| 1.00 | 20.74 | -1.35 |

| TABLE 6 | Parameters for an electrode with an outer diameter of 6.35 mm |
|----------|------------------|------------------|
| \( l \) [mm] | \( \varepsilon_s \) | \( \ln (\varepsilon_s - \varepsilon_t) \) |
| 0.00 | 3.90 | 2.84 |
| 0.01 | 4.35 | 2.81 |
| 0.02 | 4.85 | 2.78 |
| 0.03 | 5.08 | 2.77 |
| 0.04 | 5.51 | 2.74 |
| 0.05 | 5.97 | 2.71 |
| 0.10 | 7.15 | 2.63 |
| 0.15 | 8.45 | 2.53 |
| 0.20 | 9.48 | 2.44 |
| 0.25 | 10.16 | 2.38 |
| 0.30 | 10.76 | 2.33 |
| 0.40 | 12.74 | 2.11 |
| 0.50 | 13.98 | 1.95 |
| 0.60 | 15.43 | 1.72 |
| 0.70 | 16.49 | 1.51 |
| 0.80 | 17.47 | 1.26 |
| 0.90 | 18.14 | 1.05 |
| 1.00 | 18.90 | 0.74 |
| 1.20 | 19.69 | 0.27 |
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this does not affect our determination of the penetration depth, as explained below.\textsuperscript{24,25} Equation 2 can be rewritten as

\[
\varepsilon_a \varepsilon_r = (\varepsilon_a - \varepsilon_r) \exp \left( -\frac{I}{l_0} \right),
\]

which corresponds to a straight line in the semi-logarithmic plot shown in Figure 3. We obtained these straight lines from least-squares fits to the exponential function given by Equation 3 for each electrode diameter. The slope of this straight line gives the electric-field penetration depth \(l_0\). The penetration depth so obtained for each electrode diameter is listed in Table 7. The penetration depth clearly increases with increasing electrode diameter. This diameter dependence is reasonable, because the electric field lines from the central to the outer conductors are longer for electrodes with larger outer diameters.

4.2 | Human skin observed using dielectric spectroscopy

Dielectric relaxation spectra obtained from TDR measurements of the palm side of the forearm using electrodes of various diameters are shown in Figure 4. The curves show at least two relaxation processes, one in the frequency region above 10 GHz and the other below 1 GHz. Figure 4 also shows that larger dielectric constants and losses are obtained for electrodes with larger diameters.

The dielectric relaxation curves thus obtained can be described by the sum of two Cole-Cole equations:

\[
\varepsilon^* = \varepsilon_m + \frac{\Delta \varepsilon_h}{1 + (j \omega \tau_h)^\beta} + \frac{\Delta \varepsilon_l}{1 + (j \omega \tau_l)^\beta},
\]

where \(\varepsilon^*\) is the complex dielectric permittivity, \(\varepsilon_m\) is the high-frequency limit of the dielectric constant, \(\Delta \varepsilon\) is the relaxation strength, \(\tau\) is the relaxation time, \(\beta\) is the relaxation time distribution parameter, and \(h\) and \(l\) indicate the higher and lower relaxation processes, respectively. A typical result of a fit of Equation 4 to the relaxation processes obtained for the palm side of the forearm using the 2.20 mm-diameter electrode is shown in Figure 5. It is reasonable to assume that the \(h\) process results from the reorientation of free water molecules and that the \(l\) process results from a superposition of multiple factors, such as interfacial polarization, bound water, and local chain motions of macromolecules, as indicated in our previous work.\textsuperscript{20}

Figures 6 and 7 show the relationship between the relaxation strength of the \(h\) process and the thickness \(d\) of the epidermis for two examinees. We obtained the thickness of the epidermis at the various measurement sites from the literature on anatomically based thicknesses,\textsuperscript{32} as listed in Table 8. The actual thickness of the epidermis for each examinee will in fact be different. Plots of the logarithm of \(\Delta \varepsilon\) obtained for each electrode, however, showed straight

### Table 7: Penetration depth of the electric field

| \(\phi\) [mm] | 0.86 | 1.19 | 2.20 | 3.56 | 6.35 |
|-------------|------|------|------|------|------|
| \(l_0\) [mm] | 0.052 | 0.11 | 0.15 | 0.30 | 0.49 |

FIGURE 3 Approximation lines in semi-logarithmic plots for various electrodes. The symbols are the same as in Figure 2

FIGURE 4 Frequency dependence of the complex dielectric constant of human skin obtained using various probe diameters. The symbols are the same as in Figure 2

FIGURE 5 Frequency dependence of the complex dielectric constant obtained with an electrode having an outer diameter of 2.20 mm. The solid lines correspond to the two relaxation processes obtained from the fitting procedure.
lines against \( d \), as shown in Figures 6 and 7. Though the relaxation strength depends on both the examinee and the physical conditions, Figures 6 and 7 indicate that this linear dependence is common and suggest that any such influence is small compared with the difference among the measured body parts.

In addition, we found that the thickness of the epidermis was neither extremely thin nor extremely thick compared to the average value for each measured body part, even for examinees. The straight lines show the dependence on the thickness of the body part of each examinee. Figure 7 shows that the straight lines for electrodes with larger penetration depths generally have shallower slopes and larger values of the relaxation strength \( \Delta \varepsilon \) than those shown in Figure 6. This means that the contribution of dermis with high water content is smaller in Figure 6. Therefore, it is conceivable that examinee A (Figure 6) has a thicker epidermis than examinee B (Figure 7).

The vertical axes of Figures 8 and 9 represent the moisture content of the skin for each body part obtained by all electrodes, with different electric-field penetration depths \( l_0 \). Electrodes with larger \( l_0 \) values apparently probe regions with larger moisture content, since the ratio of water-rich dermis to water-poor epidermis increases with \( l_0 \). These curves show that examinee A exhibited

| Table 8 Thickness of the epidermis of different body parts, from Ref. 32 |
|-----------------------------|-------------------------------|
| Body parts                  | Thickness of epidermis [mm]   |
| Cheek                       | 0.042                         |
| Palm side of the forearm    | 0.086                         |
| Palm                        | 0.145                         |
| Sole of the foot            | 0.185                         |

**Figure 6** Relationship of the relaxation strength and the thickness of the epidermis for probes with outer diameters of 0.86, 1.19, 2.20, 3.58, and 6.35 mm [examinee A: male] [Colour figure can be viewed at wileyonlinelibrary.com]

**Figure 7** Relationship of the relaxation strength and the thickness of the epidermis for probes with outer diameters of 0.86, 1.19, 2.20, 3.58, and 6.35 mm [examinee B: female] [Colour figure can be viewed at wileyonlinelibrary.com]
measurements with various electrodes that the epidermis has a less change in water content, compared with examinee B, because of the former’s thicker epidermis. For examinee A, with a thicker epidermis, even if the penetration depth of the electrode increases, the apparent moisture content does not increase much. In other words, the present technique of dielectric spectroscopy can determine the unknown thickness of the epidermis of any body part.

Figure 10 shows the relationship between the relaxation strength of the h process and the thickness of the epidermis for the 2.2 mm-diameter electrode for four examinees. We found that for a given electrode diameter the difference in the slope depends on the examinee. This difference in the slope of the straight line expresses the difference in the thickness of the epidermis for each examinee. These results also depend on the physical condition of the day, however, although the effect is small compared to the difference between different body parts. Even if the thickness of the epidermis depends on the examinee, it is neither extremely thin nor extremely thick compared to the average value for each body part, as in Figures 6 and 7. Thus, the relative thickness of each body part exhibits a linear dependency for each examinee. Even if the thickness of the epidermis depends on the examinee, the relative thickness of each part generally offers the linear dependency, as it is neither extremely thin nor extremely thick compared to the average value of each part. These characteristic behaviors suggest a methodology for a detailed assessment of the skin using the present analysis of systematic dielectric measurements with coaxial electrodes having different penetration depths.

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