Percolation Analysis in Electrical Conductivity of Madin-Darby Canine Kidney and Caco-2 Cells by Permeation-Enhancing Agents

Makiko Washiyma, a Naoya Koizumi, a Makiko Fujii, a Masuo Kondoh, b Kiyohito Yagi, b and Yoshiteru Watanabe*. a

a Department of Pharmaceutics and Biopharmaceutics, Showa Pharmaceutical University; 3–3165 Higashitamagawagakuen, Machida, Tokyo 194–8543, Japan; and b Graduate School of Pharmaceutical Sciences, Osaka University; 1–6 Yamadaoka, Suita, Osaka 565–0871, Japan.

Received September 7, 2012; accepted November 28, 2012

The control of permeability through the paracellular route has been paid great attention to for enhanced bioavailability of macromolecular and hydrophilic drugs. The paracellular permeability is controlled by tight junctions (TJ), and claudins are the major constituents of TJ. Despite numerous studies on TJ modulation, the dynamics is not well understood, although it could be crucial for clinical applications. Here, we studied the time (t) course of electrical conductivity (Σ) in a monolayer of Madin-Darby canine kidney (MDCK) and Caco-2 cells upon treatment with modulators, the C-terminus fragments of Clostridium perfringens enterotoxin (C-CPE) and sodium caprate (C10). For C-CPE treatment, Σ remains approximately constant, then starts increasing at t=t1 (percolation threshold). For C10, on the other hand, Σ increases to 1.6–2.0 fold of the initial value, stays constant, and then starts increasing again for both MDCK and Caco-2 cells at t=t2. We find that this behavior can be explained within a framework of percolation, where Σ shows a logarithmic dependence on t−t1 with the power of μ. μ denotes the critical exponent. We obtain μ=1.1–1.2 regardless of cell type or modulator. Notably, μ depends only on the dimensionality (d) of the system, and these values correspond to those for d=2. Percolation is thus the operative mechanism for the increase in Σ through TJ modulation. The findings provide fundamental knowledge, not only on controlled drug delivery, but also on bio-nanotechnologies including the fabrication of biological devices.

Key words percolation analysis; tight junction; transepithelial electrical resistance; critical exponent; claudin; permeation-enhancing agent

Drug delivery routes include transcellular and paracellular pathways. The permeability through the former for macromolecular and hydrophilic drugs is quite low. The control of permeability through the paracellular route has thus received a great deal of attention for enhanced bioavailability.1,2) The paracellular permeability across epithelial cells is controlled principally by tight junctions (TJ), which are located at the most apical portion of cell–cell contact points.3) Claudins are the major constituents of TJ and form a strand network.4) A single TJ strand consists of a continuous array of small particles having a diameter of 5–10 nm,4) and claudins between adjacent cells form the strand through -interaction. The barrier property of these strands depends strongly on the combination of claudin types between adjacent cells,5) and hence, on cell types. In addition, TJ strands dynamically change their topology within <30 s.6) For modulating TJ barrier functions, including permeability of molecules and transepithelial electrical resistance (TER), the C-terminus fragments of Clostridium perfringens enterotoxin (C-CPE) and sodium caprate (C10) have been extensively studied as absorption enhancers. Comprehensive reviews on the molecular basis of the structures,9) as well as physiology and function10) of TJs have been made. A number of models correlating TJ structures with their barrier function have been proposed. A strand-count model11) and a binary sieving model12) are typical examples. In the former model, the TJ strand number correlates with TER under the assumption of an equal barrier function of TJ strands over a wide range of cell types and a fixed topology of the structure. The latter model consists of restrictive and non-restrictive pores in parallel, and explains solute size dependency of permeability. The random connection of permeable and less- (or non-)permeable paths is a common feature of these models. This physical view corresponds to what is called percolation on a random resistor network (RRN).

Many systems including electrical conductivity (Σ) in composites, forest fire spreading and ferromagnetism exhibit percolation.13) Percolation, for instance, Σ in a conductor/insulator binary system with the fraction of a conductor p, is characterized by its scaling relation.13) At a certain value of p (=p), termed percolation threshold in p, the interconnected resistors percolate throughout the system, and Σ thus starts increasing in a logarithmic manner, where Σ∝(p−p)μ. Note that p depends both on the dimensionality (d) and the details of the system, while μ, the critical exponent, depends only on d.13) Theoretical and experimental works as well as computer simulations have been performed and report p and μ for d and many types of lattice.13) In biological systems, the percolation network model for trafficking and signaling through the cytoskeleton has been described.14)

Despite accumulating works on TJ modulators, the dynamics or time-dependent behavior is not well understood, although it could be crucial from the viewpoint of controlled drug delivery. Treatment with C-CPE, for instance, alters the strand structure from a continuous to discontinuous array of small particles.4) This structure change is accompanied by a change in TER. In the current study, we adopt the time course of TER after treatment with C10 or C-CPE to evaluate the dynamics of TJ barrier function. Note that the time resolution of the TER experiment was better than that for permeability, enabling analysis of the dynamics of TJ modulation. The

The authors declare no conflict of interest.

© 2013 The Pharmaceutical Society of Japan
The objective of this investigation was to evaluate whether percolation is applicable to $\Sigma$ and, if so, to determine the $\mu$ and its dependency on cell type and modulator (C10 or C-CPE). This investigation is expected to further our fundamental knowledge on controlling the drug delivery rate, cell signaling and bio-nanotechnology, including fabrication of devices.

**MATERIALS AND METHODS**

**Antibodies and Materials** Mouse anti-claudin-4 as the primary antibody and goat anti-mouse immunoglobulin G (IgG) fluorescein isothiocyanate (FITC) conjugate as the secondary antibody were purchased from Invitrogen (Carlsbad CA, U.S.A.). Dulbecco’s modified Eagle’s medium (DMEM), phosphate-buffered saline (PBS) and bovine serum albumin (BSA) were from Sigma-Aldrich (St. Louis, MO, U.S.A.). Fetal bovine serum (FBS) was from SAFC Biosciences, U.S.A. Penicillin–streptomycin mixed solution (PSS), non-essential amino acids solution (MEM) and Triton-X were from Nacalai Tesque (Kyoto, Japan). Sodium caprate (C10) and formaldehyde were from Tokyo Chemical Industries (Tokyo, Japan) and Wako Pure Chemical Ind., Ltd. (Osaka, Japan), respectively.

**C-CPE Preparation** C-CPE consisting of amino acid residues 184–319 of CPE was prepared as previously reported. The C-CPE plasmid was transfected into *Escherichia coli*, resulting in BL21 (DE3) strains for the synthesis of C-CPE. The cells were harvested, resuspended, and then lysed with a sonicator. The lysates were applied to the Ni-column, eluted with imidazole, and then purified using a PD-10 column (GE Healthcare, Japan). The resulting C-CPE solution was stored at $-80^\circ$C until use.

**Cell Culture** Madin-Darby canine kidney (MDCK) and human epithelial colorectal adenocarcinoma (Caco-2) cells were cultured on a 24-well InterCell dishes (Kurabo, Osaka, Japan) at 37°C in supplemented DMEM [DMEM/FBS/PSS/MEM=500/50/5 in volume] at 95% relative humidity with 5% CO$_2$. The culture medium was replenished every day until TER to reach a constant value.

**TER Assay** The resistance was monitored with an ohm-meter, Millicell, (Millipore, Billerica, MA, U.S.A.) every 24 h until the resistance reached a constant value; the resistance of a cell monolayer was calculated by Eq. 1.

$$R_{\text{cell}}(t) = R_{\text{obs}}(t) - R_{\text{med}}$$

Here, $R_{\text{obs}}(t)$, $R_{\text{cell}}(t)$ and $R_{\text{med}}$ denote the resistance of the whole system, cell at time $t$ and that of medium, respectively. Note that $\text{TER}=A\times R_{\text{cell}}^{10}$; $A=$surface area of a single well. Once the TER reached a constant value, C10 (3 mg/ml) or C-CPE (0.01 mg/ml) was immediately added to the apical or basal compartments, respectively. A typical value of TER for MDCK and Caco-2 was 700 Ω·cm$^2$ and 250 Ω·cm$^2$, respectively. $R_{\text{cell}}(t)$ was then monitored every 10 s for 30 min at 37°C; we defined $t=0$ as the time of treatment with C10 or C-CPE. We adopted $\Sigma$ (=1/$R$) rather than TER for analyses hereafter. No deconvolution of the transmission function of the electrodes was made, since the response time was short enough to be ignored; the response time was about 1 s, while the experimental time course was 10$^3$–10$^4$ s. At least three independent experiments were performed for each case.

**Lactate Dehydrogenase (LDH) Assay** In order to evaluate cell damage, we measured LDH activity using the Cytotox 96 Assay kit (Promega, Madison, WI, U.S.A.) following the manufacture’s manual. The relative C10 or C-CPE-induced LDH release rate (LRR) for each sample was calculated by Eq. (2), where $ABS_{\Sigma}$, $ABS_{\Sigma \text{MAX}}$ and $ABS_{\Sigma \text{MIN}}$ denote the absorbance of samples after 30 min of C10 or C-CPE treatment, treated with 1% Triton-X100 and without C10 or C-CPE treatment, respectively.

$$LRR(\%) = \frac{ABS_{\Sigma} - ABS_{\Sigma \text{MIN}}}{ABS_{\Sigma \text{MAX}} - ABS_{\Sigma \text{MIN}}} \times 100$$ (2)

It was found that $LRR<5\%$ (C10) and ca. 1% (C-CPE) after 30 min of exposure. Cytotoxicity was thus ignorable within the experimental time course.

**Immunofluorescence Microscopy (IFM)** Cell monolayers cultured on an InterCell dish were fixed with 1% formaldehyde at 4°C for 3 h then incubated with 0.1% Triton X-100 for 15 min. After rinsing with PBS, the fixed cells were blocked with 5% BSA in PBS for 45 min, and then incubated with the primary antibodies for 1 h at room temperature. After rinsing with PBS several times, cells were then incubated with the secondary antibodies for 1 h at room temperature. After rinsing again with PBS, cells were carefully mounted on a glass strip, and images of these cells were taken using a bioimaging microscope (CKX41 and IMT2; Olympus, Tokyo, Japan) equipped with a CCD camera.

**THEORETICAL DEVELOPMENT**

**Equivalent Direct Current (DC) Circuit** The equivalent DC circuit is that where the paracellular conductors, consisting of the $\Sigma_{\text{TJ}}$ (TJ) and $\Sigma_{\text{LS}}$ (lateral intercellular space) in series, connect with $\Sigma_{\text{trans}}$ (transmembrane) and $\Sigma_{\text{other}}$ (other than these three) in parallel. The contribution of $\Sigma_{\text{LS}}$ could thus be neglected. Consequently, the conductivity at time $t$ is approximately given by Eq. 3.

$$\Sigma_{\text{cell}}(t) = \phi_{\text{par}} \Sigma_{\text{TJ}}(t) + \phi_{\text{trans}} \Sigma_{\text{trans}}(t) + \phi_{\text{other}} \Sigma_{\text{other}}$$ (3)

Here $\phi_{\text{par}}$, $\phi_{\text{trans}}$ and $\phi_{\text{other}}$ denote the fraction of paracellular, transcellular and that which is other than the first two, respectively.

**Analytical Model and Mathematics** As a representation of the TJ, a conductive or a less conductive path is assigned respectively to a conductor of which conductivity is $\sigma_1$ or $\sigma_2$ ($h=\sigma_2/\sigma_1<1$), therewith defining an RRN, where the fraction of the conductor having $\sigma_1$ is $p$. The barrier property of the less conductive path for various types of cells is reflected in $\sigma_2$. Based on the effective medium approximation$^{10}$ or renormalization group approach,$^{17}$ the expression for the $\Sigma$ of this RRN is given by Eqs. 4 and 5.$^{16-18}$

$$\Sigma = \sigma_1 |\Delta p|^\mu \phi_p(z)$$ (4)

$$z = h/|\Delta p|^\mu$$ (5)

Here, $\Delta p=p-p_c$; $p_c$ is the percolation threshold in $p$. $\mu$ and $s$ denote the critical exponent for $\sigma_1=0$ (insulator) and $\sigma_1=\infty$ (superconductor), respectively. $\phi_p(z)$ is the scaling function and is given by Eq. 6; the subscript± denotes the sign of $\Delta p$. 

March 2013 385
\[ \phi(x) \equiv A_1 + A_2 x + \ldots \text{ for } z \ll 1 \text{ and } \Delta p > 0 \] (6A)

\[ \phi(x) \equiv A_1 x + A_2 x^2 + \ldots \text{ for } z \ll 1 \text{ and } \Delta p < 0 \] (6B)

In the case of \( z \to 0 \), combining Eqs. 4 and 6, we obtain Eq. 7.

\[ \Sigma / \sigma_i \propto \Delta p^\theta \text{ for } \Delta p > 0 \] (7A)

\[ \Sigma / \sigma_i \propto h^{\mu/(\nu+p)} \to 0 \text{ for } \Delta p = 0 \] (7B)

\[ \Sigma / \sigma_i \propto h \times \Delta p^{-\kappa} \to 0 \text{ for } \Delta p < 0 \] (7C)

\( \Sigma \) remains approximately constant for \( \Delta p < 0 \), then starts increasing for \( \Delta p > 0 \). In the case of \( 0 < z \ll 1 \), on the other hand, \( \phi(z) \) needs a second term or more in Eq. 6A, deviating from the scaling relationships in 7A. Taking the first two terms in Eq. 6A and comparing with Eq. 7A, one can easily obtain a smaller \( \mu \) for \( 0 < z < 1 \).

**Analysis** In general, \( p \) is a function of time, \( p = f(t) \), so that the Taylor expansion around \( t = t_c \), \( \Delta t \) yields Eq. 8, i.e., \( \Delta p \) is proportional to \( \Delta t = t - t_c \).

\[ \Delta p = \frac{\partial f(t)}{\partial t} \bigg|_{t=t_c} \Delta t \] (8)

Combining Eqs. 3, 7 and 8, we obtain Eq. 9 for small \( \Delta t > 0 \) and \( z \to 0 \) if both \( \Sigma_{\text{ran}}(t) \) and \( \Sigma_{\text{other}}(t) \) are constant.

\[ \frac{\Sigma_{\text{cell}}(t)}{\Sigma_{\text{cell}}(t_c)} - 1 \propto \Sigma_{\text{TJ}}(t_c) - \Sigma_{\text{TJ}}(t_c) = \Sigma_{\text{TJ}}(t_c) \propto \Delta t^\theta \] (9)

The kinetics given in Eq. 8 governs the TJ modulation rate through \( p \) as described in Eq. 7A, since \( \mu \) is a universal number depending only on \( d \).

**RESULTS**

**Time Course of Conductance** For Caco-2 with C-PCE, \( r(t) = \Sigma_{\text{cell}}(t)/\Sigma_{\text{cell}}(0) \) remains approximately constant (ca. 1.1), then starts increasing with an inflection (Fig. 1), recovering well the behavior in Eq. 7 (Fig. 1, inserted graph). This system thus corresponds to \( z \to 0 \). The \( r(t) \) for Caco-2 with C10, on the other hand, increases with \( t \) and remains approximately constant, then increases again to show an inflection; such a two-stage behavior in TER (proportional to \( 1/\Sigma \)) has been reported before.\(^{12,19,20} \) Similar behavior is observed for MDCK for both C10 and C-PCE (Fig. 1). Note that the time at such inflection, \( t_c \), is calculated from the inflection point in the log-log graph for Caco-2 is illustrated in the inserted graph.

\[ \log(\Sigma_{\text{cell}}(t)/\Sigma_{\text{cell}}(0) - 1) \text{ vs. } \log(t) \] (Fig. 2). We herein term the early and late stage for \( t < t_c \) and \( t > t_c \), respectively. In the early stage, \( r(t) \) increases approximately to 2.0 and 1.6 on average, respectively, for MDCK; Caco-2 by the C10 treatment is comparable to values previously reported (ca. 2.0, 1.4).\(^{15,20} \) The difference in \( r(t_c) \) between MDCK and Caco-2 would be due to the difference in the sensitivity to C10.

The value of \( t_c \) tends to be larger for an extended incubation after TER to reach a constant, e.g., \( t_c \approx 360 \to 900 \) s for an additional 1-d incubation for MDCK with C10. Such an extended incubation will result in a smaller \( p \) at \( t = 0 \) due to more developed TJs, requiring a larger \( t_c \) for percolation. If the \( t_c \) merely corresponds to a response time, \( t_c \) should have been independent of the incubation time. 

**Claudin-4 Distribution** At \( t = 0 \), claudin-4 localizes at the plasma membrane for both MDCK (Fig. 4, lane 1) and Caco-2 (Fig. 4, lane 2). It should be noted that the localization of claudin-4 at the plasma membrane is not altered even by the additional 24h of incubation without the treatment by C10 or
Claudin-4 still remains at $t=3$ min after treatment with C10, while claudin-4 delocalizes and redistributes in the cytoplasm at $t=30$ min. The PDZ1 domain of ZO-1 associates with the YV motif at the C-terminal of claudin-4. The redistribution of ZO-1 observed in Caco-2 would thus result in the redistribution of claudin-4 (Fig. 4). Claudin-4 also remains at the plasma membrane at $t=3$ min after treatment with C-CPE, then becomes fuzzy (partial delocalization) at $t=120$ min and finally disappears at $t=24$ h, being equivalent to patterns previously reported for MDCK. At least the difference in the delocalization rate of claudin-4 would result in the difference in the behavior of $\Sigma_{\text{cell}}$.

**DISCUSSION**

$\mu$ and $p_c$ We obtain $\mu = 1.1–1.2$ in all cases, implying that the TJ modulation is a $d=2$ phenomenon. Since TJ modulation takes place in the paracellular space between cells, $d=2$ is not surprising. The size of the TJ strand network in depth (200–500 nm) is much smaller than the cell size or the distance between tricellular points (10–20 $\mu$m), so that the contribution from them would be negligible. The $\Sigma$ of perforated graphite sheets ($d=2$, size $= 50 \times 50$) starts increasing at $p_c = 0.6$, and the $p_c$ is close to that for site-percolation on a square lattice. It is, however, difficult in the present system to obtain an exact number of $p_c$ due to the randomness in cell shape (see also Fig. 4). The present system corresponds to

| $d=2$ | Simulation | Theory |
|------|-----------|--------|
| $d=3$ | Simulation | Theory |
| Bethe | Simulation | Theory |

Then becomes fuzzy (partial delocalization) at $t=120$ min and finally disappears at $t=24$ h, being equivalent to patterns previously reported for MDCK. At least the difference in the delocalization rate of claudin-4 would result in the difference in the behavior of $\Sigma_{\text{cell}}$.

**Table 1. The Critical Exponent, $\mu$, Obtained in This Study in Addition to Those for $d=2$ Square, $d=3$ Cubic and Bethe Lattice**

| $d=2$ | $d=3$ | $d=3$ | $d=2$ |
|-------|-------|-------|-------|
| Simulation | Simulation | Theory | Theory |
| $d=3$ | Simulation | Theory | Theory |
| Bethe | Simulation | Theory | Theory |

$\mu$ and $p_c$ We obtain $\mu = 1.1–1.2$ in all cases, implying that the TJ modulation is a $d=2$ phenomenon. Since TJ modulation takes place in the paracellular space between cells, $d=2$ is not surprising. The size of the TJ strand network in depth (200–500 nm) is much smaller than the cell size or the distance between tricellular points (10–20 $\mu$m), so that the contribution from them would be negligible. The $\Sigma$ of perforated graphite sheets ($d=2$, size $= 50 \times 50$) starts increasing at $p_c = 0.6$, and the $p_c$ is close to that for site-percolation on a square lattice. It is, however, difficult in the present system to obtain an exact number of $p_c$ due to the randomness in cell shape (see also Fig. 4). The present system corresponds to

**Fig. 3. Relationship between Log[$\Sigma_{\text{cell}}(t)/\Sigma_{\text{cell}}(t_c) - 1$] and Log($\Delta t$).**

The slope, which corresponds to $\mu$, for C10-Caco-2 and C-CPE-Caco-2 is 1.08 ($R^2=0.998$) and 1.18 ($R^2=0.981$), respectively. The solid lines are calculated from the least-squares regression analysis.

**Fig. 4. Time Course of the Distribution of Claudin-4**

MDCK (lane-1) and Caco-2 (lane-2).
the bond-percolation and the shape of the cell appears to be between square and hexagonal, so that \( p_c = 0.50 - 0.65 \).^{13}

**Finite Size Effect** As previously pointed out, the size of the TJ strand network in the depth direction is much smaller than that in the lateral direction. The system may thus be insufficient as an infinite size. The finite size effect yields smaller values for \( p_c \) and \( \mu \).^{24,25} Indeed, the \( \mu \) is slightly smaller than that for \( d=2 \) (Table 1). At \( p = p_c \), finite size scaling for \( \Sigma \) yields Eq. 10, where \( L_z \) and \( v \) denote the system size (proportional to the number of conductors) in depth and the critical exponent for the correlation length, respectively.\(^{18,24,25}\)

\[
\Sigma \propto L_z^{-0.975} = L_z^{-1}
\]

(10)

If \( L_z \) would be proportional to the quantity of claudin-4 (\( C_{\text{Cla4d}} \)), \( \Sigma \) is approximately inversely proportional to \( C_{\text{Cla4d}} \), recovering the results previously pointed out.\(^{26}\)

**Comparison of C10 and C-CPE** The following mechanisms will be active upon treatment with C10. Firstly, the expansion of the paracellular gap results in a larger \( \Sigma_{TJ} \) and \( \Sigma_{\text{trans}} \). This process includes the activation of phospholipase C (PLC), upregulation of intracellular Ca\(^{2+}\), phosphorylation of myosin light chain kinase (MLCK) and actin-myosin filament contractions.\(^{26,27}\) This is active only in the late stage, since the inhibitor of MLCK is effective only in the late stage\(^{28}\) despite the rapid elevation (within 1–2 min) of the intracellular Ca\(^{2+}\) concentration.\(^{28}\) Secondly, the delocalization of claudin-4 from the plasma membrane via the delocalization of ZO-1\(^{28}\) or the disruption of lipid rafts\(^{33}\) increases, at least in \( \Sigma_{TJ} \). Such delocalization of claudin-4 does not occur within 3 min, but can be seen within 30 min (Fig. 4), consistent with previous reports.\(^{29}\)

Lastly, the membrane perturbation increases in \( \Sigma_{\text{trans}} \) within 3 min.\(^{29}\) This time course is comparable to that seen in the present study. Although the LRR is small, and hence leads to a small increase in \( \Sigma_{\text{trans}} \), this contribution may not be ignorable, since the \( \Sigma_{\text{trans}} \) is large. Furthermore, morphology change may also affect \( \Sigma_{\text{other}} \).

The above first two observations imply that a significant increase in \( \Sigma_{TJ} \) would not be the cause for the increase in \( r(t) \) in the early stage. Based on the binary sieving model, not the pore size, but rather the number of pores is altered after C10 treatment.\(^{33}\) suggesting that \( p \) increases, but that \( \sigma_1 \) or \( \sigma_2 \) remains constant. Thus, if \( \Sigma_{TJ} \) mainly controls \( \Sigma_{cell} \) in the early stage, \( r(t) \) should have remained approximately constant as observed for C-CPE (Fig. 1); however, this was not the case at all. PLC inhibitors affect both stages.\(^{29}\) In addition, \( r(t) \) increases to 1.2–1.3 at \( t = 200 \) s, then remains almost constant up to \( t = 1200 \) s when measured at \( 4 \) °C, where kinase activity is extremely suppressed. These observations thus suggest that the increase in \( r(t) \) in the early stage will be caused at the least by the increase in \( \Sigma_{\text{trans}}(t) \) via membrane perturbation coupled with the elevation of intracellular Ca\(^{2+}\). The early stage is completed by \( t = t_4 \) since \( r(t_4) \) is approximately constant. In contrast, the C-CPE may induce less membrane perturbation, since C-CPE does not penetrate into the plasma membrane.\(^{29}\) If membrane perturbation occurred, \( r(t) \) should have been 1.6–2.0 rather than <1.1. The \( r(t) \) thus remains approximately constant in the early stage.

**Applicability and Limits** In the present study, we use MDCK and Caco-2 cell lines, which are epithelial cells from different species and organs. In addition, we use different TJ modulators having different TJ modulation mechanisms. Furthermore, the expression level of claudin isoforms in MDCK and Caco-2 is different especially in claudin-2 and -3.\(^{4,15,21,32}\) Despite such differences, \( \mu \) is found to be \( \mu = 1.1−1.2 \) regardless the cell type and TJ modulator. The \( \mu \) is thus independent of TJ opening mechanisms and claudin isoforms responsible for TJ modulation, suggesting that percolation would be a universal event in \( \Sigma \). The percolation analysis of this study would thus be applicable to (intestinal) tissue, which is a multilayer system. However, \( \mu \) for such tissue would be different from that for a cell monolayer. The conductive paths between cell layers may contribute to \( \Sigma \). In this case, the interaction between layers should be considered. The \( d \) may thus be \( d>2 \), resulting in a greater value of \( \mu \) (compare \( \mu \) for \( d=2 \), 3 in Table 1). Generally, \( d \) depends on the topology (or connection pattern) of conductive paths.

The diffusion of macromolecular drugs, including proteins and DNA, through TJs has become more important. Due to the analogy between \( \Sigma \) and diffusion coefficient (\( D \)), current percolation analysis can be applicable to the diffusion through TJ. The evaluation and control of \( \mu \) would thus be important in establishing effective drug delivery systems.

The applicability of this percolation analysis is limited under several conditions. Firstly, the scaling form Eq. 7A is not appropriate for leaky TJ, where \( h \rightarrow 1 \) (\( z < 1 \)), and the slope, \( \partial \log(\Sigma) / \partial \log(Dp) \), is no longer a constant near \( p = p_c \) and becomes smaller, as discussed previously\(^{17,18}\) (see also Eq. 6). For instance, \( \Sigma \) is a constant, and hence \( \mu = \partial \log(\Sigma) / \partial \log(Dp) = 0 \), when \( h = 1 \). In this case, detailed information on \( \Sigma_{TJ}(t_c) \) is necessary (see Eq. 9). Secondly, if \( \Sigma_{\text{trans}}(t) \) or \( \Sigma_{\text{other}}(t) \) decreases beyond \( t > t_4 \), Eq. 9 is not a good approximation, yielding a larger number of \( \mu \). In this case, the contribution from \( \Sigma_{\text{trans}}(t) \) and/or \( \Sigma_{\text{other}}(t) \) should be subtracted. This occurrence may depend on cell type, modulator and their concentration,\(^{12,15,20}\) although this was not the case in the present study.

**CONCLUSION**

The time course of electrical conductivity, \( \Sigma \), in MDCK or Caco-2 cell monolayers upon treatment with C-CPE or C10 was investigated. We found that \( \Sigma \) of TJ could be described within a framework of percolation and determined the critical exponent, \( \mu \), to be 1.1–1.2, corresponding to that for \( d=2 \) regardless of the cell type or modulator used. TJ modulation is thus a two-dimensional phenomenon, and percolation is the operative mechanism in \( \Sigma \). Due to the analogy between \( \Sigma \) and \( D \), current percolation analysis can be applicable to the diffusion through TJ and provide important knowledge especially in establishing effective drug delivery systems through the control of \( \mu \). However, the applicability of percolation analysis is limited to tight TJ strands (\( h \leq 1 \)).

**Acknowledgement** This work was supported by a Grant-in-Aid for Scientific Research C (21590178) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

**REFERENCES**

1) González-Mariscal L, Nava P, Hernández S. Critical role of tight junctions in drug delivery across epithelial and endothelial cell
layers. *J. Membr. Biol.*, 207, 55–68 (2005).

2) Salama NN, Eddington ND, Fasano A. Tight junction modulation and its relationship to drug delivery. *Adv. Drug Deliv. Rev.*, 58, 15–28 (2006).

3) Johnson LG. Applications of imaging techniques to studies of epithelial tight junctions. *Adv. Drug Deliv. Rev.*, 57, 111–121 (2005).

4) Sonoda N, Furuse M, Sasaki H, Yonemura S, Katohara J, Horiguchi Y, Tsukita S. *Clostridium perfringens* enterotoxin fragment removes specific claudins from tight junction strands: Evidence for direct involvement of claudins in tight junction barrier. *J. Cell Biol.*, 147, 195–204 (1999).

5) Furuse M, Sasaki H, Tsukita S. Manner of interaction of heterogeneous claudin species within and between tight junction strands. *J. Cell Biol.*, 147, 891–903 (1999).

6) Sasaki H, Matsu C, Furuse K, Mimori-Kiyosue Y, Furuse M, Tsukita S. Dynamic behavior of paired claudin strands within ap-posing plasma membranes. *Proc. Natl. Acad. Sci. U.S.A.*, 100, 3971–3976 (2003).

7) Kondoh M, Masuyama A, Takahashi A, Fujii M, Yonemura S, Watanabe Y, Koizumi N, Fujii M, Hayakawa T, Horiguchi Y, Watanabe Y. A novel strategy for the enhancement of drug absorption using a claudin modulator. *Mol. Pharmacol.*, 67, 749–756 (2005).

8) Tomita M, Hayashi M, Awazu S. Absorption-enhancing mechanism of sodium caprate and decanoylcarcinine in Caco-2 cells. *J. Pharmacol. Exp. Ther.*, 272, 739–743 (1995).

9) Furuse M. Molecular basis of the core structure of tight junctions. *Cold Spring Harb. Perspect. Biol.*, 2, a002907 (2010).

10) Anderson JM, Van Itallie C. Physiology and function of the tight junction. *Cold Spring Harb. Perspect. Biol.*, 1, a002584 (2009).

11) Claude P. Morphological factors influencing transepithelial permeability: a model for the resistance of the zonula occludens. *J. Membr. Biol.*, 39, 219–232 (1978).

12) Watson CJ, Rowland M, Warhurst G. Functional modeling of tight junctions in intestinal cell monolayers using polyethylene glycol oligomers. *Am. J. Physiol. Cell Physiol.*, 281, C388–C397 (2001).

13) Stauffer D, Aharony A. *Introduction to Percolation Theory*, 2nd ed., Taylor and Francis, London (1994).

14) Shafrir Y, Ben-Avraham D, Forgacs G. Trafficking and signaling through the cytoskeleton: a specific mechanism. *J. Cell Sci.*, 113, 2747–2757 (2000).

15) Sugiyabashi K, Onuki Y, Takayama K. Displacement of tight junction proteins from detergent-resistant membrane domains by treatment with sodium caprate. *Eur. J. Pharm. Sci.*, 36, 246–253 (2009).

16) Kirkpatrick S. Percolation and conduction. *Rev. Mod. Phys.*, 45, 574–588 (1973).

17) Costa UMS, Tsallis C, Schwachheim G. Conductivity of a square-lattice bond-mixed resistor network. *Phys. Rev. B Condens. Matter*, 33, 510–514 (1986).

18) Clerc JP, Giraud G, Laugier JM, Luck JM. The electrical conductivity of binary disordered systems, percolation clusters, fractals and related models. *Adv. Phys.*, 39, 191–309 (1990).

19) Söderholm JD, Olaison G, Peterson KH, Franzén LE, Lindmark T, Wirén M, Tagessson C, Sjödahl R. Augmented increase in tight junction permeability by luminal stimuli in the non-inflamed ileum of Crohn’s disease. *Gut*, 50, 307–313 (2002).

20) Lindmark T, Kimura Y, Artursson P. Absorption enhancement through intracellular regulation of tight junction permeability by medium chain fatty acids in Caco-2 cells. *J. Pharmacol. Exp. Ther.*, 284, 362–369 (1998).

21) Itoh M, Furuse M, Morita K, Kubota K, Saitoh M, Tsukita S. Direct binding of three tight junction-associated MAGUKs, ZO-1, ZO-2, and ZO-3, with the COOH termini of claudins. *J. Cell Biol.*, 147, 1351–1363 (1999).

22) Last BJ, Thouless DJ. Percolation theory and electrical conductivity. *Phys. Rev. Lett.*, 27, 1719–1721 (1971).

23) Hsu HP, Huang MC. Percolation thresholds, critical exponents, and scaling functions on planar random lattices and their duals. *Phys. Rev. E Stat. Phys. Plasmas Fluids Relat. Interdiscip. Topics*, 60 (6 Pt A), 6361–6370 (1999).

24) Sahimi M, Hughes BD, Screen L, Davis HT. Critical exponent of percolation conductivity by finite-size scaling. *J. Phys. C Solid State Phys.*, 16, L521–L527 (1983).

25) Cardy J. *Scaling and Renormalization in Statistical Physics*, Cambridge Lecture Notes in Physics 5, Cambridge University Press, Cambridge (1997).

26) Van Itallie C, Rahner C, Anderson JM. Regulated expression of claudin-4 decreases paracellular conductance through a selective decrease in sodium permeability. *J. Clin. Invest.*, 107, 1319–1327 (2001).

27) Hayashi M, Sakai T, Hasegawa Y, Nishikawahara T, Tomioka H, Iida A, Shimizu N, Tomita M, Awazu S. Physiological mechanism for enhancement of paracellular drug transport. *J. Control. Release*, 62, 141–148 (1999).

28) Tomita M, Hayashi M, Awazu S. Absorption-enhancing mechanism of EDTA, caprate, and decanoylcarcinine in Caco-2 cells. *J. Pharm. Sci.*, 85, 608–611 (1996).

29) Tomita M, Hayashi M, Horie T, Ishizawa T, Awazu S. Enhancement of colonic drug absorption by the transcellular permeation route. *Pharm. Res.*, 5, 786–789 (1988).

30) Takahashi A, Kondoh M, Masuyama A, Fujii M, Mizuguchi H, Horiguchi Y, Watanabe Y. Role of C-terminal regions of the C-terminal fragment of *Clostridium perfringens* enterotoxin in its interaction with claudin-4. *J. Control. Release*, 108, 56–62 (2005).

31) McGlothin J, Padfield PJ, Burt JP, O’Neill CA. Ochratoxin A increases permeability through tight junctions by removal of specific claudin isoforms. *Am. J. Physiol. Cell Physiol.*, 287, C1412–C1417 (2004).

32) Dong N, Tomita M, Hayashi M. Absorption enhancement effect of acylcarcinines through changes in tight junction protein in Caco-2 cell monolayers. *Drug Metab. Pharmacokinet.*, 26, 162–170 (2011).