Acta Biochimica Polonica

Vol. 43 No. 2/1996

339–348

QUARTERLY

Lecture delivered at the International Conference on "New Frontiers in Cell and Molecular Biology" held in Warsaw (Poland) on October 2–7, 1995

Applications of impedance spectroscopy in biochemistry and biophysics*

Andreas Janshoff, Joachim Wegener, Claudia Steinem, Manfred Sieber and Hans-Joachim Galla

Institut für Biochemie, Westfälische Wilhelms-Universität Münster, Wilhelm-Klemm-Str. 2, 48149 Münster, Germany

Key words: impedance spectroscopy; gramicidin; solid supported lipid layer; cell monolayer; transepithelial resistance

The present study is intended to demonstrate the application of impedance spectroscopy to two very different fields of biophysical research. The core component of our measuring setup is a self-constructed continuous wave impedance spectrometer together with special measuring chambers which are individually designed for the systems under investigation. We directed our attention towards: i) the investigation of solid supported lipid bilayers in general, especially systems which are suitable for protein reconstitution such as dimethyldioctadecylammonium bromide (DODAB) immobilized onto a gold electrode, precovered with a negatively charged monolayer of 3-mercaptopropionic acid. Impedance spectroscopy allows to study the stability, the thickness and the electrode coverage of those artificial membranes as well as the observation of ion transport mediated by ionophores like gramicidin D incorporated into a DODAB-bilayer. ii) The characterization of the passive electrical properties of epithelial and endothelial cell monolayers in general and especially the determination of their transepithelial (transendothelial) electrical resistances as a measure for epithelial barrier function. From impedance spectra, as reported here, we are able to follow the formation and modulation of cell layer permeability to small ions.

Impedance spectroscopy (IS) is a powerful and rather new technique to investigate the electrical properties of a variety of different materials which may be ionic, semiconducting or even insulating [1]. It provides information about both the materials' bulk phase (e.g., conductivity, dielectric constant) as well as their inner and outer interfaces (e.g., capacitance of the interfacial region and derived quantities). The method is based on measuring the frequency dependent impedance of the electrochemical system of interest and its analysis with the help of equivalent circuits modelling the electrical properties of the system. A linear

*This work has been financially supported by the Deutsche Forschungsgemeinschaft (SFB 310 / B10) and by the Bundesministerium für Bildung und Forschung (BMBF). A. Janshoff is a recipient of an award of the Fonds der Chemischen Industrie, J. Wegener is supported by a scholarship of the Studienstiftung des Deutschen Volkes and C. Steinem is supported by the Graduiertenförderung des Landes Nordrhein Westfalen.

Abbreviations: DODAB, dimethyldioctadecylammonium bromide; IS, impedance spectroscopy; TER, transepithelial (transendothelial) electrical resistance; ES, electrochemical system; NLSQ, non-linear-least-square-fit; MPA, 3-mercaptopropionic acid; CPE, constant phase element.

current to voltage relationship for the electrochemical system is an indispensable prerequisite for a meaningful impedance analysis. There are basically two different approaches to acquire impedance data that differ with respect to the excitation signal. The first and most common technique is to measure the impedance in the frequency domain by applying a single frequency sinusoidal voltage of small amplitude to the system and recording the corresponding current. Repetition of this procedure for a set of different frequencies provides a frequency spectrum of the system's impedance. The second approach makes use of a transient excitation signal that is applied to the electrochemical system. The system's response is registered in the time domain and subsequently transformed to the frequency domain by Fourier-Transform algorithms providing the frequency dependent impedance of the system as well.

One interesting and important application of impedance spectroscopy in biochemistry and biophysics is the characterization of solid supported lipid bilayers on a conductive surface. Because of their excellent long-term stability supported lipid bilayers are gaining widespread interest as useful model systems to study basic interaction mechanisms responsible for the structure and function of the biological membrane [2, 3]. Moreover the application of supported lipid bilayers in molecular biosensors including ligand-receptor binding may be advantageous [4, 5]. AC impedance analysis allows to study the formation process of solid supported bilayers and to investigate their long-term-stability and their stability towards experimental conditions like temperature, pH, etc. Moreover ion transport through these bilayers mediated by ion carriers or channel-proteins is accessible by IS.

Here we present a study concerning an artificial membrane which is electrostatically attached onto a negatively charged monolayer of 3-mercaptopropionic acid. This lipid bilayer was used as a lipid environment for the well-known ion-channel gramicidin. Two different equivalent circuits were applied to evaluate the impedance spectra obtained for the immobilized bilayer as well as for the ion transport of monovalent cations through the incorporated gramicidin channels [6, 7].

Besides its value in studying artificial bilayer systems, impedance analysis is also a very useful tool in investigations concerning biological membranes as represented by living cells in tissue culture. We applied this technique to epithelial and endothelial cell monolayers in order to examine their passive electrical properties. The predominant function of both epithelial and endothelial cell layers is to build up and maintain a highly selective permeability barrier between two compartments of different chemical composition. The blood-brain-barrier, built up by brain capillary endothelial cells, and the blood-cerebrospinal-fluid-barrier, formed by epithelial cells of the choroid plexus, are two examples for the extreme significance of the epithelial and endothelial barrier properties [8, 9]. One physical parameter to quantify the barrier function of a given epithelial or endothelial cell layer — as far as ion permeation is concerned — is the electrical resistance across this cell layer [10], which is commonly called the transepithelial (transendothelial) electrical resistance (TER). We here present AC impedance analysis as a technique to determine TERs of epithelial or endothelial cells cultured directly on gold surfaces that are used as measuring electrodes [11].

IMPEDANCE SPECTROSCOPY

Impedance spectrometer

Impedance data were acquired using a self-constructed continuous wave spectrometer developed in our laboratories [12]. The experimental setup for impedance analysis is illustrated in Fig. 1a. Controlled by a personal computer a small sinusoidal AC voltage with constant amplitude ($\hat{U}_0$ = 60 mV), supplied by a frequency generator (Tektronix, FG5010), was applied to the resistor R_0 and the electrochemical system (ES) in series. U_0 and the voltage drop across the electrochemical system U_υ were recorded by the impedance analyzer as a function of frequency and converted to the magnitude of the amplitude ratio $|U_\upsilon(\upsilon)/U_0|$, ranging between 0 and 1 by definition. The principle of this voltage divider is demonstrated in Fig. 1b. We furthermore acquired the phase angle ϕ (υ) between the two voltages U_0 and U_υ as a function of frequency. The impedance of the electrochemical system was recorded in the frequency range from 1 s^{-1} to

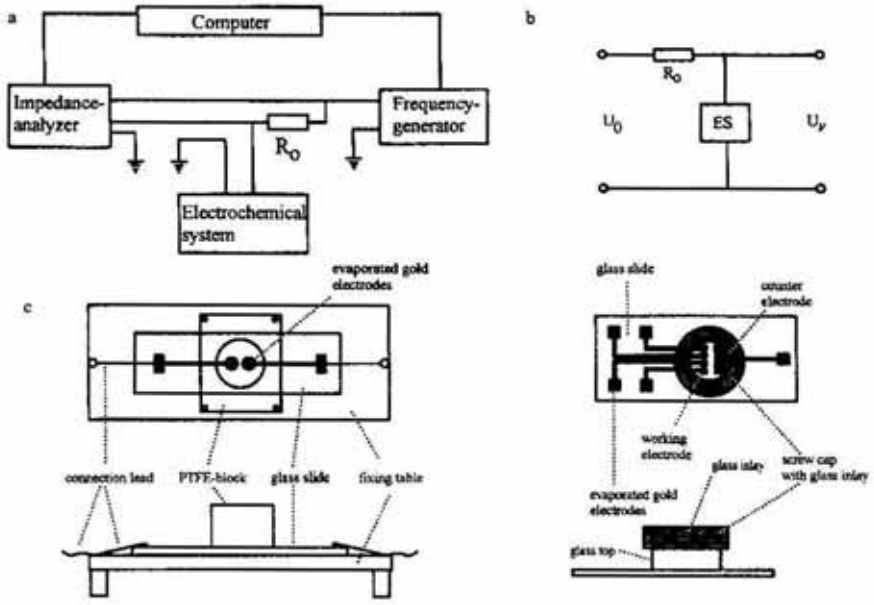

Fig. 1. a. Experimental setup used for impedance analysis. b. Principle of impedance measurement. U_0 denotes the voltage drop across the serial arrangement of the electrochemical system and the resistor R_0, supplied by the frequency generator. U_υ is the voltage drop across the electrochemical system. c. Measuring chambers used for our investigations of either thin organic films (left) or cell monolayers (right).

100000 s^{-1}. Acquisition of impedance data takes about 10 min recording 14 data points per frequency decade.

Evaluation of the recorded data

We confined ourselves to the frequency dependent magnitude of amplitude ratios for impedance analysis, as the phase angle spectrum does not provide any further information about the electrochemical system but is linked to lower signal to noise ratios. For a quantitative analysis of the electrical parameters of the system under investigation appropriate equivalent circuits were chosen to model each experiment. In order to apply these equivalent circuits to the recorded data it is first necessary to transform the assumed complex impedance of the network $Z(\upsilon)$ to the corresponding transfer function U_υ/U_0 according to equation (1):

$$\frac{U_\nu}{U_0}(\nu) = \frac{Z(\nu)}{Z(\nu) + R_0} \tag{1}$$

Exemplarily equation (2) describes the magnitude of the transfer function for an equivalent circuit consisting of a resistor R_m and a capacitor C_m in parallel combined with a resistor R_e in series (compare Fig. 2):

$$\left|\frac{U_\nu}{U_0}(\nu)\right| = \sqrt{\frac{(R_m + R_e)^2 + (2\pi\nu)^2 R_m^2 R_e^2 C_m^2}{(R_0 + R_m + R_e)^2 + (2\pi\nu)^2 (R_0 + R_e)^2 R_m^2 C_m^2}} \tag{2}$$

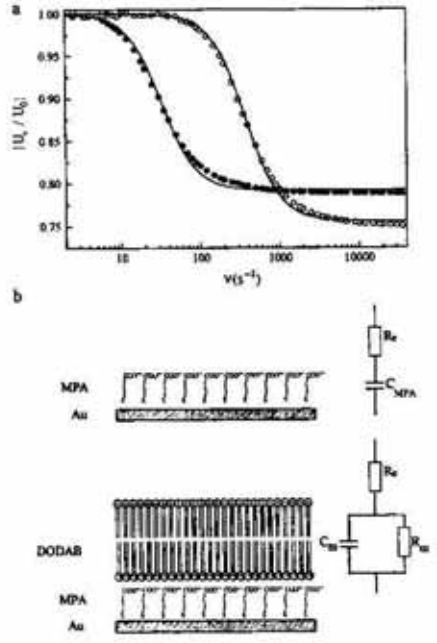

Fig. 2. a. Impedance spectra of a MPA-monolayer on gold (●) and a DODAB-bilayer immobilized onto the MPA-monolayer (○).
The MPA-monolayer was formed by a self-assembly process of the thiol-molecules onto the gold surface. The addition of DODAB-vesicles in 10 mM Tris, pH 8.6 led to a bilayer formation by fusing of the vesicles onto the negatively charged surface. The solid lines represent the results of the fitting procedure. b. Schematic diagram of the MPA-monolayer chemisorbed to the gold surface and the DODAB-bilayer immobilized onto the MPA-monolayer. The equivalent circuits used for impedance data analysis are given next to the schematic figure.

The parameters of the transfer function are fitted to the recorded data by means of a non-linear-least-square-fit (NLSQ) according to the Levenberg-Marquardt-algorithm [13].

Measuring chambers

The different electrode configurations for either the investigation of thin organic films (left) or cell monolayers (right) are shown in Fig. 1c. Gold electrodes formed by evaporation onto glass substrates were used in both applications.

Two equally designed gold electrodes were chosen to study the properties of thin organic films which are immobilized onto the gold substrates. The measuring cell consists of a PTFE-chamber with a volume of 4 ml sealed with a KALREZ®-sealing ring (Busak & Shamban GmbH & Co., Stuttgart, F.R.G.).

In order to obtain impedance data of epithelial or endothelial cell monolayers, we developed special measuring chambers, that allow to culture the different cell types under ordinary culture conditions (37°C, 5% CO_2, 95% air, humidified atmosphere) and to record impedance data without any disturbance of the culture. Therefore a glass top supplied with a screw cap is glued to the glass substrate by a non-cytotoxic silicon glue. The gold films used as measuring electrodes are prepared thin enough to be transparent and therefore allow to observe the cultured cells on top of the gold electrodes with common light microscopes. Each chamber was equipped with four small sized working electrodes together with a common, large sized counter electrode in order to determine local inhomogeneities of the electrical parameters. A computer controlled relay switch allows to change from one working electrode to another.

RESULTS

Solid supported lipid bilayers

Different preparation techniques were developed in the last ten years to form solid supported lipid bilayers. We only investigated membranes immobilized onto gold surfaces. All preparation techniques are based on the well-known chemisorption of thiol-functionalized molecules onto gold.

One example of a solid supported bilayer onto gold is given in Fig. 2. The first monolayer of 3-mercaptopropionic acid (MPA) was obtained by exposing the freshly prepared gold electrodes to a 10 mM MPA-solution in water. An impedance spectrum of the MPA-monolayer is shown in Fig. 2. In order to analyze the obtained data, we chose a simple equivalent circuit consisting of a capacitor C_{MPA} and a resistor R_e in series; C_{MPA} represents the electrical behavior of the monolayer, whereas R_e includes the ohmic behavior of the bulk electrolyte. The fit provides a capacitance C_{MPA} of 8.1 $\mu F/cm^2$. A pH-value of 8.6 was necessary to get fully deprotonated MPA. The negatively charged surface allows to fuse positively charged vesicles of dimethyldioctadecyl-ammonium bromide (DODAB) to the surface. The fusion of the DODAB-vesicles onto the MPA-layer was a rapid process and was finished after one hour yielding a bilayer electrostatically bound on the MPA-monolayer and only with small amount of defects.

Fig. 2 also shows the obtained impedance spectrum of a DODAB-bilayer on the preformed MPA-monolayer. The equivalent circuit used for data analysis (Fig. 2) consists of a parallel arrangement of the capacitor C_m and the resistor R_m, attributed to the electrical properties of the bilayer, completed by a serial resistor R_e for the conductivity of the bulk. The membrane resistance R_m is determined to about 1 $M\Omega \cdot cm^2$. The capacitance of the membrane C_m is calculated to 0.84 $\mu F/cm^2$ assuming that the capacitance of the preformed MPA monolayer does not significantly contribute to the total capacitance of the system.

In order to establish a solid supported system containing a channel-forming ionophore within the lipid bilayer matrix, the well-known channel forming polypeptide, gramicidin D from *Bacillus brevis* was chosen. This peptide forms a pore of 4 Å in diameter, which is selective for monovalent cations. Gramicidin D was reconstituted into the DODAB-vesicles and fused to the negatively charged MPA-monolayer as described above. In a concentration of 1:100 (protein:lipid) the peptide did not influence the bilayer-formation significantly. In the absence of monovalent cations the capacitance for the DODAB-bilayer with 1 mol% gramicidin D reached a value of $C_m = 0.92$ $\mu F/cm^2$, fitted with the equivalent circuit shown in

Fig. 2. The membrane resistance R_m was in the range of 1 MΩ·cm^2. The impedance spectra of this gramicidin doped DODAB-bilayer changed significantly in the presence of monovalent cations. Fig. 3 shows the impedance spectra of the bilayer in the presence of 13 mM and 31 mM CsCl, respectively. The simple equivalent circuit from Fig. 2 is not applicable to the obtained data. To improve the parameter fit we had to introduce an additional subcircuit representing the ion-transport through the channel. It consists of a resistor R_m which describes the ion flux through the membrane, the capacitance C_m of the membrane and a parallel RC-subcircuit (R_{pt}, C_{pt}) representing the situation at the interface. Because of the increasing admittance due to the ion-transport the impedance contribution of the MPA-monolayer is no longer neglectable and therefore forces to introduce the capacitance C_{MPA} in series to the described network.

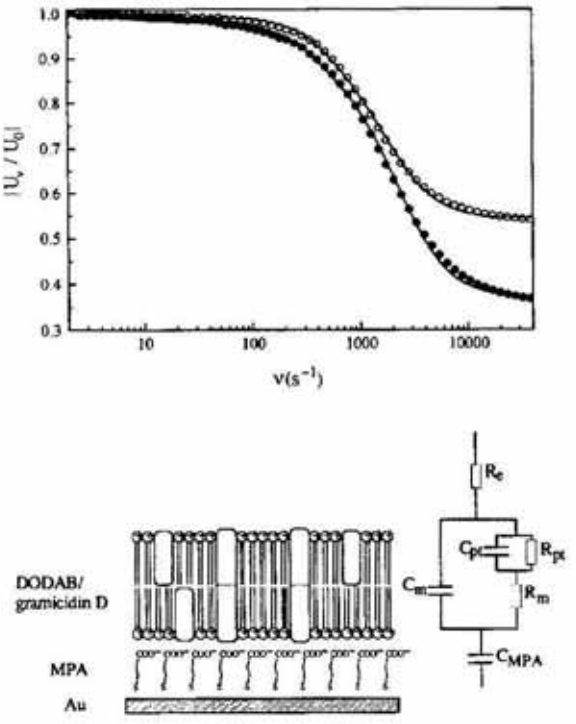

Fig. 3. Impedance spectra of a gramicidin D (1 mol%) doped DODAB-bilayer immobilized onto the MPA-monolayer in the presence of 13 mM (○) and 31 mM (●) CsCl.

The solid lines represent the results of the fitting procedure with the given equivalent circuit leading to the following parameters: C_m = (0.95 ± 0.02) μF/cm^2, R_m = (500 ± 25) Ω·cm^2, C_{pt} = (0.45 ± 0.02) μF/cm^2, R_{pt} = (7100 ± 700) Ω·cm^2 for the bilayer in the presence of 13 mM CsCl and C_m = (1.17 ± 0.02) μF/cm^2, R_m = (330 ± 10) Ω·cm^2, C_{pt} = (1.15 ± 0.05) μF/cm^2, R_{pt} = (2100 ± 200) Ω·cm^2 for the bilayer in the presence of 31 mM CsCl. The capacitance of the MPA-monolayer C_{MPA} was kept constant to C_{MPA} = 8.1 μF/cm^2 during the fitting procedure. The resistor R_0 was adjusted to 1 kΩ.

Corresponding experiments with tetramethylammonium chloride, which does not permeate through the gramicidin D channels because of its enlarged radius (data not shown), were carried out. This ion did not influence the impedance of the membrane.

Epithelial and endothelial cell monolayers

Impedance analysis was carried out for primary cultured endothelial cells from porcine brain microvessels, epithelial cells from porcine choroid plexus and the epithelial cell line MDCK. Moreover, we investigated human skin fibroblasts and smooth muscle cells from porcine aorta that are known not to exhibit barrier properties due to the absence of special cell-cell contacts, the so-called tight junctions.

Fig. 4a shows typical impedance spectra of the gold electrodes with (○) and without leaky epithelial cells (●) on top of their surfaces. The spectrum of the uncovered gold electrode is analyzed using an equivalent circuit that consists of a capacitor C_{el} and a resistor R_{bulk} in series. The capacitor represents the dielectric properties of the electrode/electrolyte interface whereas the resistor is mainly attributed to the conductivity of the bulk electrolyte. Quantitative analysis reveals about 10 μF/cm^2 for the capacitance of the electrode interface, which is significantly lower than predicted from Gouy-Chapman theory. We got several experimental indications that the diminished capacitance is due to the adsorption of medium ingredients to the gold surface, most notably the adsorption of cysteine and other thiol-compounds *via* self-assembly processes. Furthermore we assume that the adsorption of medium ingredients to the gold surface provides the hydrophilicity necessary for cell attachment, since it is well known that gold surfaces are normally of hydrophobic nature and therefore unsuitable as culture surfaces.

Considering the spectrum of the cell covered gold electrode in Fig. 4a (○), it is obvious from the appearance of the spectrum that the presence of the cell layer on top of the electrodes alters the frequency dependent imped-

ance of the electrochemical system in the frequency range from 100 to 10000 s^{-1}. This spectrum is analyzed using the equivalent circuit depicted in Fig. 4b. Within this model the capacitor C_{el} and the resistor R_{bulk} are attributed to the electrode/electrolyte interface and the bulk electrolyte, respectively, as discussed above. The cell monolayer is therefore represented by the parallel arrangement of the resistor $R_{c.l.}$ and the capacitor $C_{c.l.}$. The given equivalent circuit

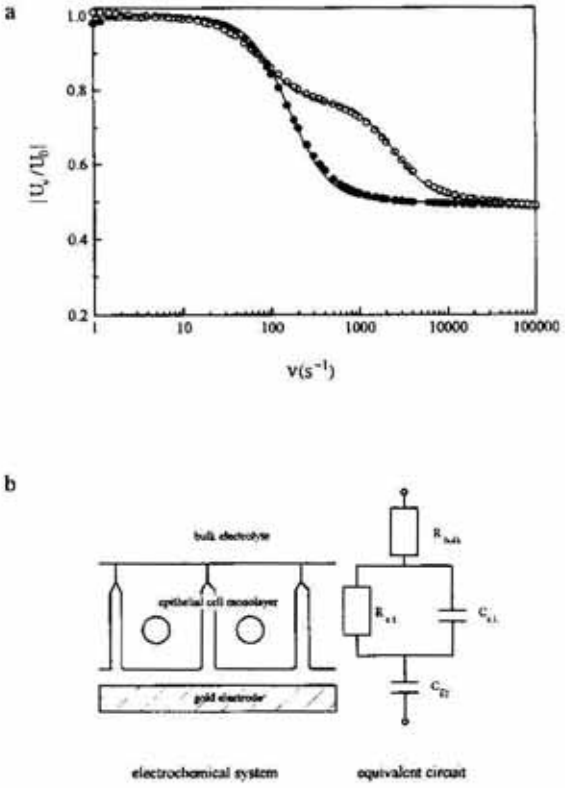

Fig. 4. a. Typical impedance spectra of cell-free gold electrodes (●) and gold electrodes covered with a confluent monolayer of leaky epithelial cells (○) (MDCK-II) in ordinary cell culture medium as bulk electrolyte.

The solid lines represent the results of the fitting procedures of the corresponding equivalent circuits. For the cell-free gold electrodes: C_{el} = (10.7 ± 1.1) μF/cm^2 and R_{bulk} = (248 ± 2) Ω. For the cell-covered electrode: C_{el} = (9.4 ± 1) μF/cm^2, R_{bulk} = (248 ± 2) Ω, $R_{c.l.}$ = (112 ± 12) Ω·cm^2, $C_{c.l.}$ = (1.3 ± 0.2) μF/cm^2. The resistance of the bulk electrolyte is not normalized to the surface area as it depends on the height of the electrolyte solution in the measuring chamber. The resistor R_0 was adjusted to 250 Ω. b. Interpretation of the equivalent circuit used for data analysis. The capacitor $C_{c.l.}$ and the resistor $R_{c.l.}$ in parallel represent the passive electrical properties of the cell monolayer on top of the electrodes. The resistor $R_{c.l.}$ is regarded to be equivalent to the transepithelial resistance as described in the literature.

is the simplest model to fully describe the characteristics of the obtained spectra using a minimum number of parameters. In conclusion of our experiments with high and low resistant cell types as well as experiments that are commonly accepted to predictably affect TERs of a cell monolayer, we interpret the resistor $R_{c.l.}$ to be equivalent to the transepithelial resistance [8]. The spectrum in Fig. 4a is typical for "leaky" epithelia and endothelia with specific resistances in the range of 50 to 200 Ω·cm^2. We found this type of spectrum for MDCK-II cells, epithelial cells form porcine choroid plexus and endothelial cells from porcine brain capillaries. The results of equivalent circuit modelling revealed individual TERs for these cell types in accordance with published data as detailed in Table 1. MDCK-I cells on top of the gold electrodes altered the frequency dependent impedance of the electrochemical system in the frequency range from 100 to 10000 s^{-1} more strongly than the other cell types (data not shown). These cells have recently been reported to have TERs of more than 2000 Ω·cm^2 and therefore are classified to belong to the "tight" epithelia. Quantitative analysis of these impedance spectra provides TER values in the range of 1000 to 3000 Ω·cm^2 in good agreement with published data (compare Table 1).

Impedance analysis not only reveals the resistance across the cell layer $R_{c.l.}$ but also its capacitance $C_{c.l.}$. Without going into details, it is interesting to note that the capacitances of epithelial cell layers (1–3 μF/cm^2) are significantly larger than those obtained for endothelial cells (0.5–0.8 μF/cm^2). It remains to be elucidated what are the main factors influencing the monolayers' capacitances.

Impedance spectra of human skin fibroblasts on top of the electrodes are very similar to the spectra of the uncovered electrodes (not shown) and therefore these cell types are hardly detectable by impedance spectroscopy. These cell monolayers are not able to prevent ion permeation through the paracellular shunt due to the absence of tight junctions that are capable to occlude the intercellular clefts and thus they do not build up considerable electrical resistances. In terms of equivalent circuit modelling, the capacitor $C_{c.l.}$ is more or less short cut by the very low resistance of $R_{c.l.}$.

Fig. 5a illustrates the time development of impedance spectra for MDCK-II cells starting

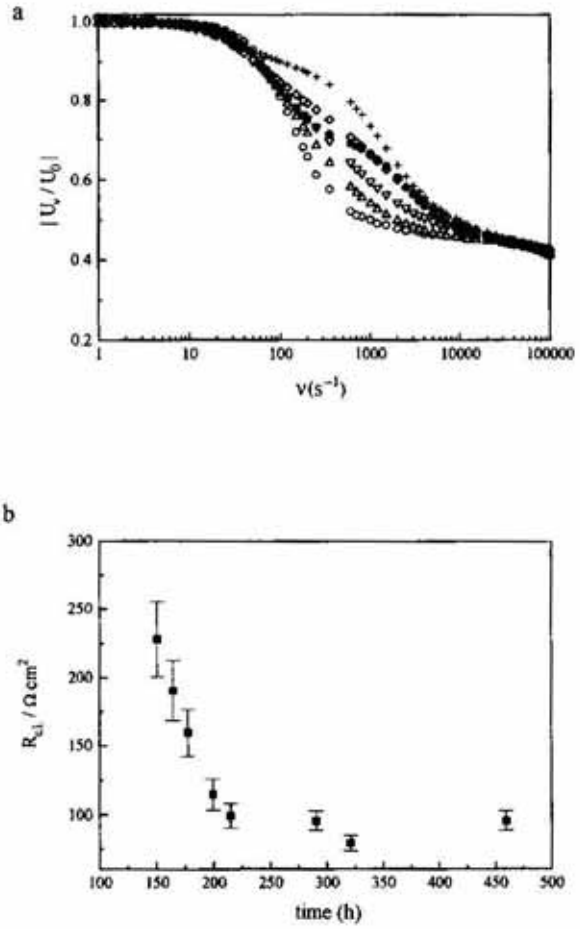

Fig. 5. a. Time development of impedance spectra for MDCK-II cells from seeding to confluence and subsequent in the culture.
The time course illustrates the establishment of a confluent monolayer on top of the electrodes. Increasing culture times are marked by the following symbols: (○) 3 h, (△) 64 h, (▽) 76 h, (◇) 135 h, (+) 150 h, (●) 321 h. b. Time course of the parameter $R_{c.l.}$ as deduced from the spectra of confluent cell monolayers.

Table 1
Transepithelial (transendothelial) resistances $R_{c.l.}$ as determined with our technique (IS) compared to literature data

Cell type	$R_{c.l.}$ (IS) / Ω·cm²	TER (literature) / Ω·cm²
MDCK-I	400–3500	2500–5000 [14]
MDCK-II	40–100	50–70 [14]
Choroid plexus epithelial cells	80–170	99 ± 15 [15]
Porcine brain endothelial cells	30–80	65 ± 17 [16]

shortly after seeding a very diluted cell suspension into the measuring chamber. The typical spectrum for confluent monolayers consisting of two distinct frequency dependent regions is continuously established during cell growth. A confluent monolayer has been formed after about 150 h in culture (dependent on the seeding density). As the equivalent circuit, discussed above, is only a meaningful model for confluent monolayers, we can evaluate only those spectra for culture times longer than 150 h. The parameter $R_{c.l.}$ as it is deduced from fitting these spectra is traced against the culture time in Fig. 5b. After reaching a maximum of about 220 Ω·cm² the resistance decreases to a steady state value of 90 to 100 Ω·cm² that remains constant for more than ten days in culture. This time course of TERs is typical for MDCK-II cell monolayers and has already been reported [17]. This experiment exemplarily validates our interpretation of the equivalent circuit.

DISCUSSION

Impedance spectroscopy was introduced as a powerful tool to determine TERs of immobilized epithelial cells onto gold as well as the electrical behavior of artificial solid supported membranes. It should also be pointed out that impedance spectroscopy is valuable to study ion transport within both systems. Useful information of several details concerning the process of penetration and permeation of ions are accessible by fitting the parameters of appropriate equivalent circuits to the recorded data.

Solid supported lipid bilayers

Impedance spectroscopy is well established to detect defects in chemisorbed or physisorbed thin organic films on solid supports. In our case we used this method to determine the electrode coverage of an electrostatically bound DODAB-bilayer on a chemisorbed monolayer of MPA. Considering equation (3), arising from the fact that in the case of holes in the bilayer the capacitance of the MPA-layer is in parallel to the defect-free membrane fragment, it can be easily deduced that small amounts of holes in the coverage yield in an increased overall capacitance of the film:

$$C_m = \Theta C_{dfb} + (1-\Theta) C_{MPA} \qquad (3)$$

C_m denotes the measured capacitance of the DODAB-bilayer, Θ the electrode coverage of the bilayer, C_{MPA} the capacitance of the preformed MPA-layer and C_{dfb} is the calculated capacitance of the defect-free bilayer (dfb) assuming that the bilayer behaves as a plate condenser:

$$C_{dfb} = \varepsilon_0 \varepsilon_r \frac{A}{d} \quad (4)$$

In equation (4) A abbreviates the electrode area, d is the thickness of the adsorbed film (about 4 nm), ε_0 denotes the dielectric permittivity of free space ($8.854 \cdot 10^{-12}$ As·V^{-1}·m^{-1}), ε_r denotes the dielectric permittivity of the bilayer relative to free space (2.4). The low value of ε_r arises from the unpolar alkyl chains, the dominating part of the molecule with respect to its length. The specific capacitance C_{dfb} may be calculated to 0.53 μF/cm^2. Inserting this value for C_{dfb} in equation (3) together with C_{MPA} = 8.1 μF/cm^2 the electrode coverage of the DODAB-bilayer on MPA is calculated to 95.9 %. Thus, the electrode coverage is sufficiently high with respect to the possible observation of ion-transport through the bilayer.

The reconstitution of the channel-forming peptide gramicidin D in DODAB-bilayers has several advantages. First of all gramicidin D reconstitutes into DODAB-vesicles exhibiting mainly channel conformation as shown by CD-measurements (data not shown) [6]. Moreover the presence of the peptide does not disturb the DODAB-bilayer formation. The impedance spectra of DODAB bilayers doped with gramicidin D cannot be fitted by the simple equivalent circuit shown in Fig. 2. In Fig. 3 we present an equivalent circuit, that describes the data well. In the presence of CsCl the capacitance C_m of the DODAB bilayer with incorporated gramicidin D increases perhaps due to a higher dielectric permittivity in the membrane phase arising from the cation within the lipid bilayer or processes concerned with the lipid-water interface. In addition the membrane resistance as well as the impedance of the subcircuit (R_{pt}, C_{pt}) decreases with increasing concentrations of the monovalent cation Cs^+ in the bulk phase. The selectivity of the ion-transport through the bilayer could be demonstrated by the use of tetramethylammonium chloride which does not penetrate gramicidin D channels.

Epithelial and endothelial cell monolayers

Up to now the determination of transepithelial resistances was limited to cell monolayers cultured on water permeable supports as for example filter inserts made from porous membranes that divide the culture dish in two compartments. These methods call for sandwich-arrangements of the measuring electrodes on opposite sides of the monolayer. Impedance analysis of gold electrodes covered with epithelial or endothelial cell monolayers allows to determine their electrical parameters on a non-permeable substrate. Therefore, conditions cells are normally exposed to in ordinary culture dishes are maintained in the presented technique. The good agreement of our results compared to literature data, as detailed in Table 1, indicates that it is of no significant influence on the TER values whether the cells are cultured on permeable or nonpermeable supports — at least for those cell types investigated in the present study.

The most popular technique to study TERs of cultured monolayers applies DC currents across a cell covered filter insert and detects the corresponding voltage drop. The resistance of the cell layer may therefore be calculated using Ohm's law including a correction for the resistance of the empty filter insert and the conductivity of the bulk. Using impedance spectroscopy as described above, transepithelial resistances are determined directly without the necessity of the mentioned corrections since the resistance of the bulk is separately determined within equivalent circuit modelling. The independence of control measurements is one reason for the improved sensitivity of the impedance technique. With respect to the equivalent circuit given in Fig. 4 it is obvious that the cell layer's passive electrical properties are also reflected by the parameter $C_{c.l.}$ Therefore, the impedance technique provides more than one independent parameter as a measure for the cell layer's ion permeability.

Some authors [18] recently proposed to use empirical instead of ideal impedance elements to describe the impedance characteristics of the interface between cell surface and bulk electrolyte (here modelled as a simple capacitor). A constant phase element (CPE) is such an empirical impedance element and it is characterized by an impedance of $Z = 1/(i \cdot \omega \cdot A)^n$. The

parameters A and n represent the specific properties of the CPE. Values for the parameter n range between 0 and 1 and are discussed to reflect the fractal nature of the interface between cell surface and bulk phase. Replacing the capacitor $C_{c.l.}$ in our equivalent circuit by such a CPE generally improves the quality of the parameter fits indicating better agreement between model and experiment. But as long as a precise physical model for the CPE and its parameters is still lacking, one has to be careful to interpret the results of the parameter fit. Therefore we prefer to use solely ideal impedance elements to model the electrochemical system.

Using more complex equivalent circuits to simulate epithelial or endothelial cell monolayers, as described in the literature [19], it is possible to get very detailed information about the electrical properties of the cell layer in terms of resistances and capacitances of the two plasma membrane domains and the intercellular space. This technique is of great advantage in investigations concerning the regulation of epithelial and endothelial barrier function. However, these complex electrical networks are not applicable to our data since they call for impedance measurements with microelectrodes that are able to determine local — probably even intercellular — impedance data.

The generous support of Degussa AG (Frankfurt/Main, F.R.G.) with gold for the electrode preparation is gratefully acknowledged. The authors are very much indebted to Dipl.-Ing. W. Willenbrink for his expertise in all sorts of electronic and computer techniques and to Dr Frederick Höhn for his careful revision of the manuscript.

REFERENCES

1. MacDonald, J.R. (1987) *Impedance spectroscopy*. John Wiley & Sons, New York.
2. Kalb, E., Frey, S. & Tamm, L.K. (1992) Formation of supported planar bilayers as receptive layers for biosensors with electrical detection. *Biochim. Biophys. Acta* **1103**, 307–316.
3. Plant, A.L. (1993) Self-assembled phospholipid/alkanethiol biomimetic bilayers on gold. *Langmuir* **9**, 2764–2767.
4. Stelzle, M. & Sackmann, E. (1989) Sensitive detection of protein adsorption to supported lipid bilayers by frequency-dependent capacitance measurements and microelectrophoresis. *Biochim. Biophys. Acta* **981**, 135–142.
5. Terrettaz, S., Stora, T., Duschl, C. & Vogel, H. (1993) Protein binding to supported lipid membranes: Investigation of the cholera toxin-ganglioside interaction by simultaneous impedance spectroscopy and surface plasmon resonance. *Langmuir* **9**, 1361–1369.
6. Steinem, C., Janshoff, A., Ulrich, W.-P., Sieber, M. & Galla, H.-J. (1996) Impedance analysis of supported lipid bilayer membranes: A scrutiny of different preparation techniques. *Biochim. Biophys. Acta* **1297**, 169–180.
7. Stelzle, M., Weissmüller, G. & Sackmann, E. (1993) On the application of supported bilayers as receptive layers for biosensors with electrical detection. *J. Phys. Chem.* **97**, 2974–2981.
8. Joó, F. (1993) The blood-brain-barrier *in vitro*: the second decade. *Neurochem. Int.* **23**, 499–521.
9. Spector, R. & Johanson, C.E. (1989) The mammalian choroid plexus. *Sci. Amer.* **261**, 48–53.
10. Claude, P. (1978) Morphological factors influencing transepithelial permeability: A model for the resistance of the *Zonula occludens*. *J. Membr. Biol.* **39**, 219–232.
11. Wegener, J., Sieber, M. & Galla, H.-J. (1996) Impedance analysis of epithelial and endothelial cell monolayers cultured on gold surfaces. *J. Biochem. Biophys. Methods* (in press).
12. Ulrich, W.-P. (1995) *Die Impedanzspektroskopie als Methode zur Untersuchung festkörperunterstützter Lipidschichten und deren Wechselwirkung mit Proteinen*. Dissertation, Universität Münster, Germany.
13. Bevington, P.R. (1969) *Data Reduction and Error Analysis for the Physical Science*. McGraw-Hill Book Company, New York.
14. Stevenson, B.R., Anderson, J.M., Goodenough, D.A. & Mooseker, M.S. (1988) Tight junction structure and ZO-1 content are identical in two strains of Madin-Darby canine kidney cells which differ in transepithelial resistance. *Cell Biol.* **107**, 2401–2408.
15. Southwell, B., Duan, W., Alcorn, D., Brack, C., Richardson, S.J., Köhrle, J. & Schreiber, G. (1993) Thyroxin transport to the brain: Role of protein synthesis by the choroid plexus. *Endocrinology* **132**, 2116–2126.
16. Erben, M., Decker, S., Franke, H. & Galla, H.-J. (1995) Electrical resistance measurements on cerebral capillary endothelial cells: A new

technique to study small surface areas. *J. Biochem. Biophys. Methods* **30**, 227–238.

17. Griepp, E.B., Dolan, W.J., Robbins, E.S. & Sabatini, D.D. (1983) Participation of plasma membrane proteins in the formation of tight junctions by cultured epithelial cells *J. Cell Biol.* **96**, 693–702.
18. Bao, J.-Z., Davis, C.C. & Schmuckler, R.E. (1993) Impedance spectroscopy of human erythrocytes: System calibration and nonlinear modelling. *IEEE Trans. Biomed. Eng.* **40**, 364–378.
19. Kottra, G. & Frömter, E. (1984) Rapid determination of intraepithelial resistance barriers by alternating current spectroscopy. II. Test of model circuits and quantification of results. *Pflügers Arch.* **402**, 421–432.