

Dielectric Analysis of Epithelial Monolayers Grown on Gelatin Balls

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Received August 25, 1989

MDCK cells derived from the dog kidney were cultured on gelatin balls (1–2 mm in diameter) to form a monolayer covering the whole surface of each ball. Spherical monolayers thus formed were subjected to dielectric measurements over a frequency range 100 Hz to 10 MHz. The dielectric behavior was found to be sensitive to the monolayer formation and the results are as follows. A dielectric dispersion, not detectable immediately after cell plating, appeared when the entire spherical surface had been covered with a monolayer. The magnitude of dielectric dispersion increased, in the subsequent culture, with an increasing tightness of intercellular junctions in the monolayer, and reached a final value after 1–2 day culture. The dielectric dispersion obtained at the final stage was analyzed based on a single shell model that a conducting core (a gelatin ball in this case) is covered with a thin shell (a monolayer) less able to conduct. The mean value of the monolayer capacity ($1.6 \mu\text{Fcm}^{-2}$) is in agreement with that obtained for the monolayers cultured on planar supports.

KEY WORDS: Dielectric dispersion/ Interfacial polarization/ Epithelial monolayer/ Cultured cell/ Monolayer capacity/ MDCK

INTRODUCTION

A number of studies have been reported on the dielectric behavior of animal organs at radio and microwave frequency ranges [1, 2]. However, none of these have been successful in estimating the electrical parameters of the constituent cells from the observed dielectric parameters because of morphological complexity. Even simple organs, such as the gall bladder, are made up of three primary tissues, i.e., epithelium, connective tissue and muscle cell layer, each of which further contains different kinds of cells interacting in various manners. It thus seems clear that in order to establish the electrical models for such organs we must have a knowledge of the dielectric properties of isolated primary tissues.

It is, however, difficult to completely separate each primary tissue from an organ by dissection. Our strategy is, therefore, to use cell culture techniques which can construct a simple "tissue" from a homogeneous population of cells. In this paper, we study the dielectric properties of an epithelial cell monolayer cultured on gelatin balls.

MATERIALS AND METHODS

Cell culture

MDCK cells, derived from the dog kidney, were cultured in Dulbecco's modified

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Eagle medium (DMEM) supplemented with 5% fetal bovine serum and 100 mg/l kanamycin at 37°C.

Preparation of gelatin balls

Stable gelatin balls 1–2 mm in diameter were prepared by the method described in a previous paper [3]. A tuberculin syringe fitted with a needle with a 90° cut tip was filled with a warm 10% gelatin solution. A careful discharge of the content into air formed a gelatin droplet, which was held *in situ* for 2–3 min until gelation completed. The droplet was cut off the needle and then transferred into a test tube containing a 2.5% glutaraldehyde solution to reinforce the droplet's mechanical stability. The gelatin balls thus obtained were thoroughly washed with deionized water and stored at 4°C.

Formation of spherical monolayers

The gelatin balls sterilized in 70% ethanol for 1–2 hr were equilibrated with culture medium for 30 min, followed by incubation in a cell suspension (10^5 – 10^6 cells/ml) for 1 hr at 37°C to allow the cells to settle down on the gelatin surface. These balls were transferred into a siliconized petri dish containing a culture medium and were incubated at 37°C until the monolayer formation was completed.

Dielectric measurements

The dielectric behavior of a single gelatin ball with or without a cell monolayer in suspension was studied with an experimental apparatus described in a previous paper [3]. Briefly, the apparatus (Fig. 1) comprises two flow-through type cells that are interconnected by polyethylene tubing for circulating medium. The specimen was loaded in

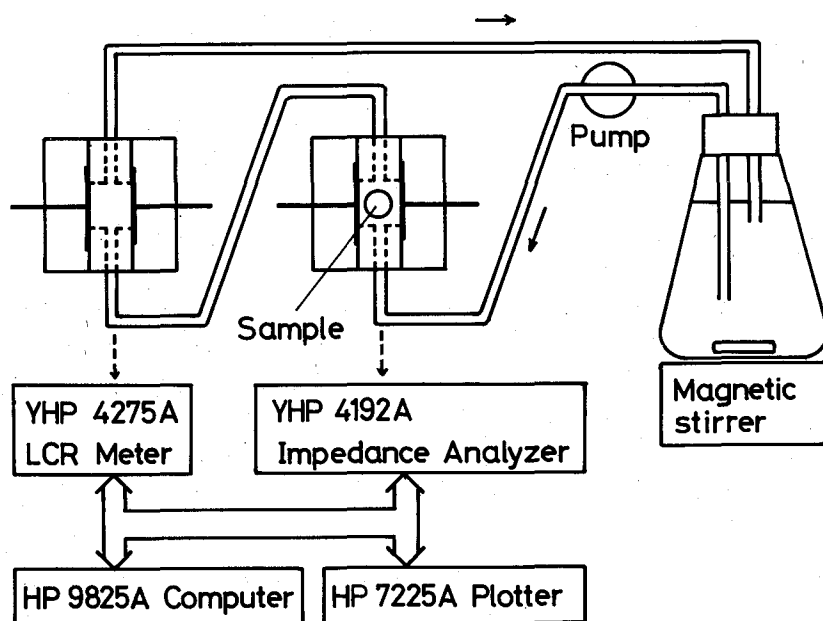


Fig. 1. Experimental arrangement for permittivity measurements. Two permittivity cells, one for sample suspension and the other for medium, are interconnected with polyethylene tubing for circulating medium.

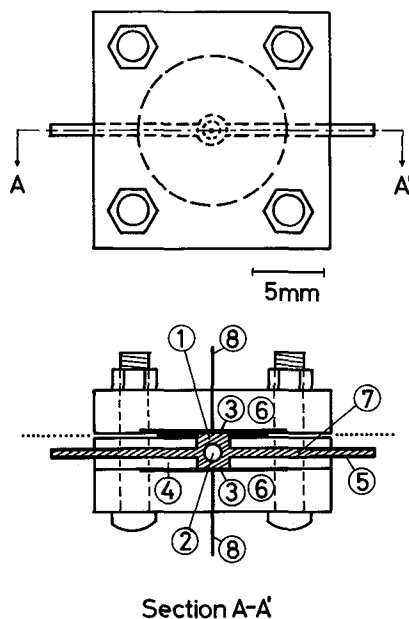


Fig. 2. Diagram of the permittivity cell used for one-ball suspension. (1) sample cavity, (2) sample, (3) platinized Pt-disc electrodes, (4) lucite spacer, (5) stainless steel tubing, (6) lucite block, (7) conduit for circulation of medium, and (8) connecting leads to the impedance analyzer. The cell is separable into two parts along the dotted line for loading a sample into the cell cavity.

one of the cells as shown in Fig. 2, and its equivalent capacitance and conductance were measured with an HP-4192A Impedance Analyzer. The other cell, which was connected to an HP-4275A LCR Meter, was used for monitoring the capacitance and conductance of the circulating medium during measurements.

Correction for residual inductance arising from the measurement cells and their leads was carried out by Schwan's method [4]. The effective volume of the sample cell cavity was estimated to be 6.3 mm^3 by the method described previously [3].

RESULTS AND DISCUSSION

Formation of monolayers on gelatin balls

The MDCK cells were cultured on a gelatin ball 1–2 mm in diameter, and the process of the cell-monolayer formation was examined by phase contrast microscopy (see Fig. 3). One hour after cell-plating (stage B), the cells were still spherical and no sign of monolayer formation was observed. After 7–8 hr culture, the ball was covered with the cells leaving no free surface, but the intercellular junctions did not appear to be tight enough at this stage (stage C). After 1–2 day culture, the monolayer was almost confluent and the cell contour became unclear (stage D), indicating a formation of tight junction between cells. The monolayer-covered gelatin ball at this final stage is termed the MDCK/gelatin ball.

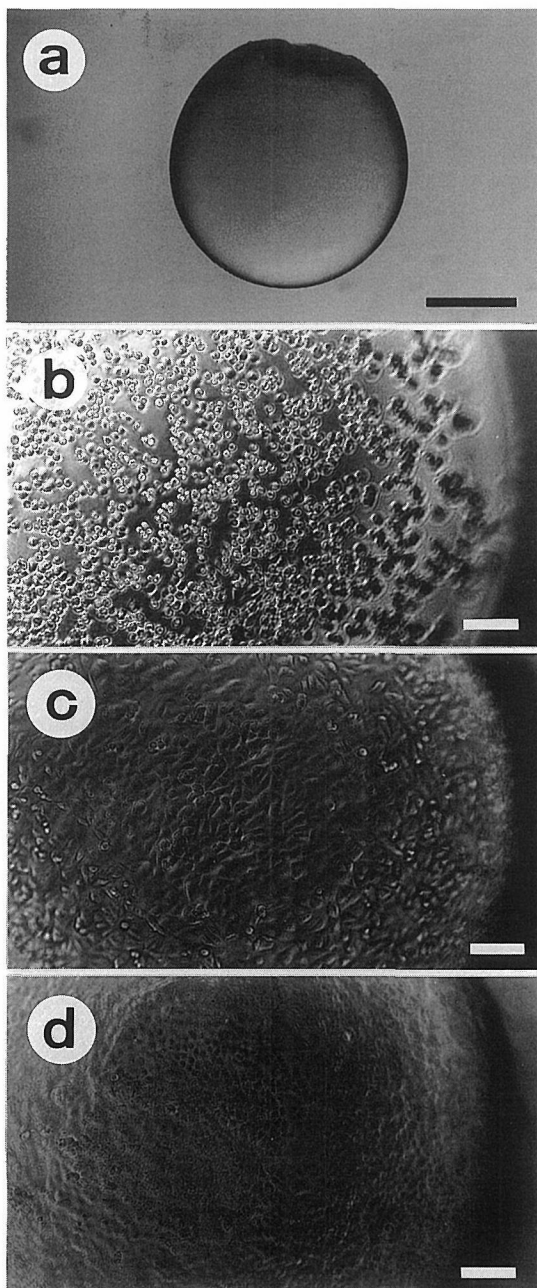


Fig. 3. Development of MDCK cell monolayer cultured on a gelatin ball, as revealed by phase-contrast microscopy. A gelatin ball prior to cell-plating (a), 1 hr after cell-plating (b), after 7–8 hr culture (c), and after 5 day culture (d). Scale bars: 0.5 mm for (a); 100 μ m for (b)–(d).

Dielectric monitoring of monolayer formation

The dielectric behavior of a cell-plated gelatin ball drastically changed during the development of cell monolayers. Figure 4 shows the dielectric dispersion profiles obtained from a ball at several stages corresponding to those in Fig. 3. No dielectric dispersion, except for electrode polarization, was found at stages A and B (prior to and immediately after cell-plating, respectively). At stage C (after 7–8 hr culture), a dielectric dispersion appeared around 50 kHz. Dispersion magnitude increased in the subsequent cultures and finally reached about 10^5 dielectric unit at stage D (after 1 day culture).

This result indicates that the dielectric behavior is sensitive to the degree of monolayer formation, i.e., the surface occupancy of the ball by the cells and the tightness of the intercellular junctions.

Volume fraction dependence of dielectric dispersion

We examined volume fraction dependence of the dielectric dispersion to establish an electrical model for the MDCK/gelatin balls (of stage D). Figure 5 shows dielectric dispersion profiles obtained with MDCK/gelatin balls of various sizes, and their complex plane plots are illustrated in Fig. 6. The dielectric parameters, extracted from the

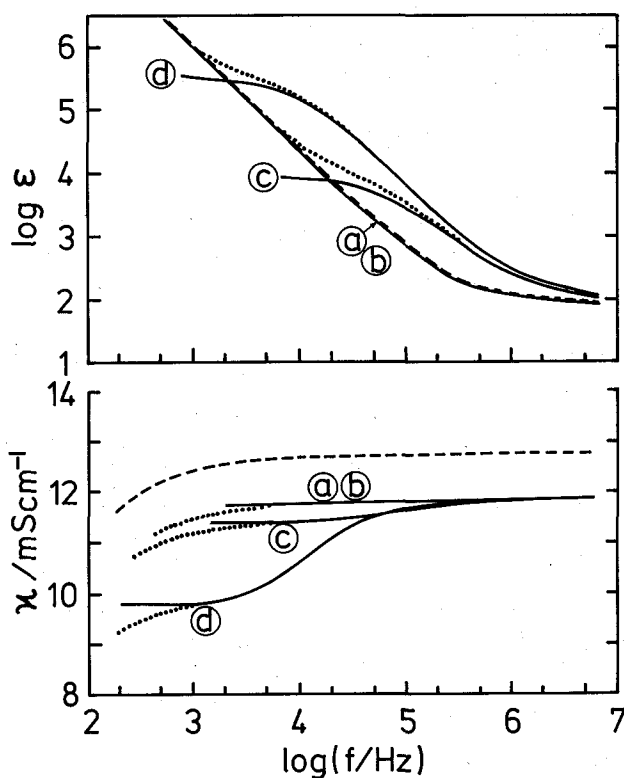


Fig. 4. Developmental changes of the dielectric dispersion profile of one cell-plated gelatin ball. Curves a–d represent the stages referred in Fig. 3. Broken lines indicate data for medium alone; dotted lines uncorrected; solid line for corrected for electrode polarization.

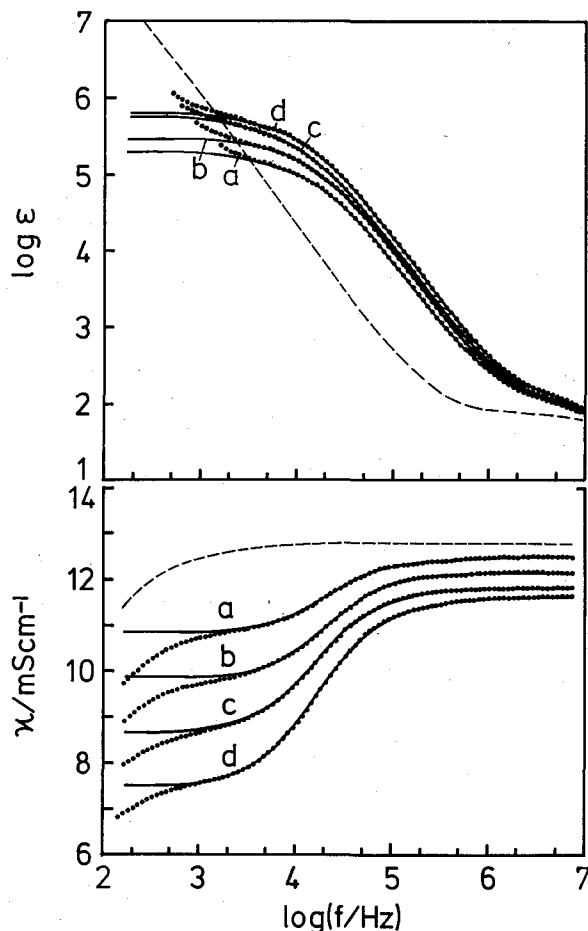


Fig. 5. Volume fraction dependence of the dielectric dispersion. Volume fractions are (a) 0.10, (b) 0.17, (c) 0.25 and (d) 0.32. Broken line indicates medium alone; dotted lines, uncorrected; solid lines, corrected for electrode polarization.

complex plane plots, changed systematically with increasing volume fraction (or ball volume); i.e., an increase in the limiting permittivity at low frequencies (ϵ_l) and concomitant decreases in the limiting conductivities at both high and low frequencies (κ_h and κ_l).

In Fig. 7, the ratio of κ_h to κ_a (the conductivity of medium) is plotted as a function of the volume fraction Φ obtained by morphometry. An approximately linear relationship was found between κ_h/κ_a and Φ . This relationship is very similar to that obtained with gelatin balls without cell monolayers (naked gelatin balls). For naked gelatin balls, the conductivity κ of the suspension, which is independent of frequency, is expressed as

$$\kappa/\kappa_a = \frac{2(1-\Phi) + (1+2\Phi)\kappa_i/\kappa_a}{(2+\Phi) + (1-\Phi)\kappa_i/\kappa_a}, \quad (1)$$

where κ_i is the conductivity of gelatin balls.

On the other hand, the volume fraction dependence of κ_i/κ_a coincided with that of

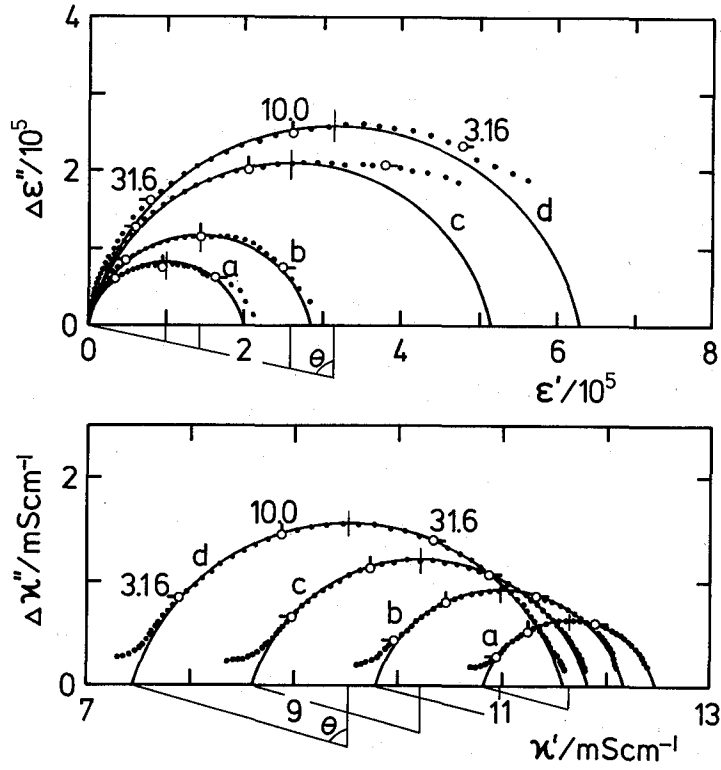


Fig. 6. Complex permittivity and conductivity plane plots of the data in Fig. 5. The vertical bars indicate characteristic frequencies. Number on each point refers to marker frequency in kHz

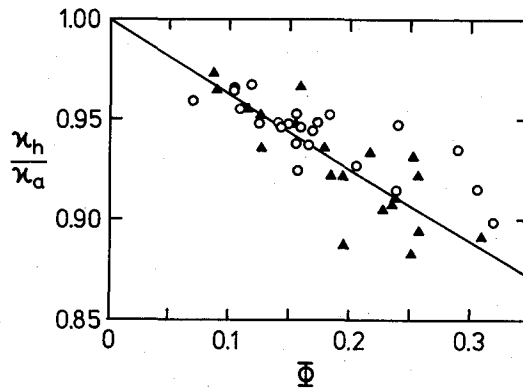


Fig. 7. Plots of κ_h/κ_a , as a function of volume fraction, for MDCK/gelatin balls (\circ), and naked gelatin balls (\blacktriangle). The volume fraction was calculated from the ball volume obtained by morphometry. The solid line was calculated from eq. 1 with $\kappa_r/\kappa_a = 0.66$.

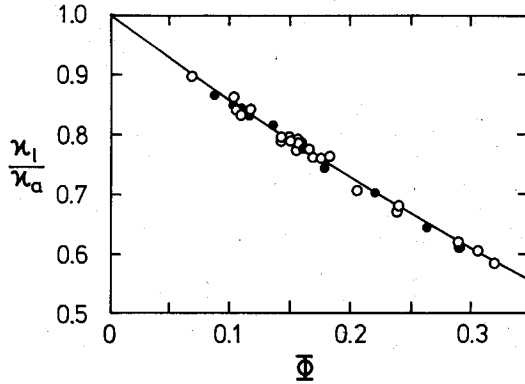


Fig. 8. Plots of κ_i/κ_a , as a function of volume fraction, for MDCK/gelatin balls (\circ), and glass beads (\bullet). The volume fraction was calculated from the ball volume obtained by morphometry. The curve was calculated from eq. 2.

the κ/κ_a obtained with non-conducting glass balls (see Fig. 8). In this case the relationship is given by eq. 2 that is derived from eq. 1 for $\kappa_i/\kappa_a \ll 1$.

$$\kappa/\kappa_a = \frac{2(1-\Phi)}{2+\Phi}. \quad (2)$$

On the basis of these results, the cell monolayer can be regarded as a poorly conducting thin layer, which is short-circuited at high frequencies. Therefore, the single-shell model shown in Fig. 9 is a plausible electrical model for the MDCK/gelatin balls. This model has been used for membrane-bounded spheres, such as spherical bilayer lipid membranes [5], microcapsules [6], and biological cells [7].

Analysis based on the single-shell model

In the single-shell model (Fig. 9), the homogeneous shell (of complex permittivity ϵ_s^*) of thickness d and the core phase (of ϵ_i^*) of radius R correspond to the monolayer and the gelatin ball, respectively. Here, complex permittivities are defined as: $\epsilon^* = \epsilon - j\kappa/\omega \in_v$; ϵ is relative permittivity, κ is conductivity, $j = \sqrt{-1}$, $\omega = 2\pi f$, f is frequency.

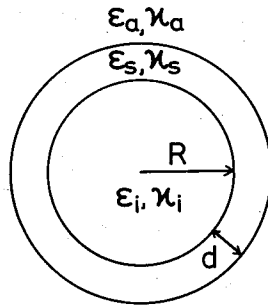


Fig. 9. A dielectric model for the MDCK/gelatin ball. ϵ , relative permittivity; κ , conductivity; R , inner radius; d , shell thickness. Subscripts a , s , and i denote suspending medium, shell phase (or monolayer) and core phase (gelatin ball), respectively.

Epithelial Monolayer Grown on Gelatin Ball

Table 1. Phase parameters estimated for MDCK-covered and naked gelatin balls.

	No. Exp.	$C_s/\mu\text{Fcm}^{-2}$	κ_i/κ_a	Φ_e/Φ_m
MDCK/gelatin ball	26	1.6 ± 0.05	0.68 ± 0.01	0.99 ± 0.01
naked gelatin ball	24		0.66 ± 0.01	

Values are expressed as mean \pm S.E. The parameter κ_i/κ_a of naked gelatin balls was calculated from eq. 1. Φ_e is the volume fraction calculated from eq. 12. Φ_m is morphometric volume fraction defined by $\Phi_m = \frac{4}{3}\pi R^3/V$, where V is the cavity volume of the measurement cell and R is ball radius.

cy, and ϵ_v is the permittivity of free space. The equivalent complex permittivity of the shelled sphere (ϵ_p^*) is given by

$$\epsilon_p^* = \epsilon_s^* \frac{2(1-v)\epsilon_s^* + (1+2v)\epsilon_i^*}{(2+v)\epsilon_s^* + (1-v)\epsilon_i^*}, \quad (3)$$

where $v = (R/(R+d))^3$. When the shelled sphere is suspended in a continuous medium of complex permittivity ϵ_a^* , the complex permittivity of the suspension (ϵ^*) is given by

$$\epsilon^* = \epsilon_a^* \frac{2(1-\Phi)\epsilon_a^* + (1+2\Phi)\epsilon_p^*}{(2+\Phi)\epsilon_a^* + (1-\Phi)\epsilon_p^*}, \quad (4)$$

where Φ is volume fraction.

Since for the MDCK/gelatin ball we can assume that $\kappa_s/\kappa_a \ll 1$, $\kappa_s/\kappa_i \ll 1$ and $d/R \ll 1$, eqs.3 and 4 are approximately rewritten as

$$\epsilon^* = \epsilon_h + \frac{\epsilon_l - \epsilon_h}{1 + j\omega\tau} + \frac{\kappa_l}{j\omega\epsilon_v}. \quad (5)$$

This equation predicts a single relaxation, of which dielectric parameters (ϵ_h , ϵ_l , κ_l , and τ) are related to phase parameters (ϵ_a , ϵ_s , κ_i , κ_a , and Φ) as:

$$\epsilon_h \simeq \epsilon_a, \quad (6)$$

$$\epsilon_l \simeq \frac{9C_s R \Phi}{(2+\Phi)^2 \epsilon_v}, \quad (7)$$

$$\kappa_l \simeq \kappa_a \frac{2(1-\Phi)}{2+\Phi}, \quad (8)$$

$$\tau \simeq RC_s \left(\frac{1}{\kappa_i} + \frac{1}{\kappa_a} \frac{1-\Phi}{2+\Phi} \right), \quad (9)$$

where C_s is the shell capacity defined as $C_s = \epsilon_s/d$. In addition, the limiting conductivity at high frequencies κ_h is given by

$$\kappa_h \simeq \kappa_a \frac{(1+2\Phi)\kappa_i + 2(1-\Phi)\kappa_a}{(1-\Phi)\kappa_i + (2+\Phi)\kappa_a}. \quad (10)$$

Equations 7, 8 and 10 may be further rewritten as:

$$C_s \simeq \frac{\epsilon_l \epsilon_v (2 + \Phi)^2}{9R\Phi}, \quad (11)$$

$$\Phi \simeq \frac{2(\kappa_a - \kappa_l)}{2\kappa_a + \kappa_l}, \quad (12)$$

$$\kappa_i \simeq \kappa_a \frac{\kappa_h(2 + \Phi) - 2\kappa_a(1 - \Phi)}{-\kappa_h(1 - \Phi) + \kappa_a(1 + 2\Phi)}. \quad (13)$$

Using eqs. 11, 12 and 13, we can calculate C_s , Φ and κ_i from the observed dielectric parameters ϵ_l , κ_l , and κ_h .

The results of the calculation are summarized in Table 1. The mean value of the monolayer capacity was $1.6 \mu\text{Fcm}^{-2}$, being in agreement with that of planar monolayers cultured on a permeable support ($1.8 \mu\text{Fcm}^{-2}$ obtained from ac measurements by Asami et al. [8], $1.4 \mu\text{Fcm}^{-2}$ from dc transient measurements by Cereijido et al. [9]). The value of κ_i/κ_a ($=0.68$) is consistent with that of naked gelatin balls. The value of Φ_e calculated from eq. 12 is very close to the value of morphometric volume fraction Φ_m , which supports the assumptions that $\kappa_s/\kappa_a \ll 1$ and $\kappa_s/\kappa_i \ll 1$.

CONCLUSION

The present results with the MDCK/gelatin ball demonstrate that the MDCK monolayer is regarded as a thin layer less able to conduct. This model may be applicable to many epithelial cell layers covering animal organs. Similar dielectric analyses coupled with cell culture techniques are underway at present to establish the electrical models of muscle cell layers and connective tissues.

ACKNOWLEDGMENTS

We wish to thank Dr. Y. Kinoshita and Mrs. T. Ichinowatari, Kochi Medical School, for helpful discussion and expert technical assistance, respectively.

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