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Analysis of Effect of Radiation Exposure on Cultured Cells using Electrical Cell-substrate Impedance Sensing (ECIS) Method

N. GODA[1,*], Y. YAMAMOTO[1], T. NAKAMURA[1], T. MARUYAMA[1], T. KUSUHARA[1], S. MOHRI[2], N. KATAOKA[3], F. KAJIYA[3]

[1] *Faculty of Health Sciences, Okayama University Medical School Okayama, Japan*
[2] *Department of Cardiovascular Physiology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences Okayama, Japan*
[3] *Department of Medical Engineering, Kawasaki Medical School Kurashiki, Japan*

A mathematical model for the micro-dynamics of cultured cells measured with the ECIS (Electrical Cell-substrate Impedance Sensing) system that can separately evaluate the cell-to-cell distance and the cell-to-substrate distance is proposed. For wide applications of this method, mathematical models considering various types of cells and confluent conditions were constructed. Using ECIS™, frequency the characteristics of 25 Hz to 60 kHz of impedance of HUVEC (human umbilical vein endothelial cells), BAEC (bovine aortic endothelial cells), in the pre-confluent condition and the full confluent condition of each cell were measured. A mathematical model of the micro-dynamics of the cultured cells measured with ECIS from 1 kHz to 10 kHz, which was the most interesting frequency range for the micro-dynamic analysis in the ECIS methods is proposed. The evaluation method of the cell-to-cell distance (A) and the cell-to-substrate distance (h) could be improved. In the application, we investigated the effect of the X-ray radiation exposure from 1 Gy to 100 Gy on the cultured cells BAEC using the ECIS system. Impedance changes could be confirmed by exposure to 100 Gy. The X-ray stimulation of 100 Gy resulted in a significant increase of the value of the cell-to-cell distance (A).

Keywords: ECIS method, bio-electrical impedance, cultured cell modeling, micro-motion of cultured cells, effect of radiation exposure

1. Introduction

A mathematical model of micro-dynamics for cultured cells measured with the ECIS (Electrical Cell-substrate Impedance Sensing) system that can separately evaluate the cell-to-cell distance and the cell-to-substrate distance [1–3] is proposed. For wide

* Correspondence to: Noriko Goda, 2-5-1 Shikata-cyo Okayama, 700-8558 Japan, e-mail: goda@md.okayama-u.ac.jp

applications of this method, we constructed mathematical models considering certain types and confluent conditions. In these models, the polarization impedance of the electrolyte medium interface is constant phase angle, and its magnitude decreases the frequencies of the negative power function. Cell impedance, mainly the cell membrane impedance is formed by the equation of Cole-Cole dispersion function, in which the system of the relaxation time is continuously distributed over a wide range.

New parameters S_A, S_h are introduced for evaluating the micro-motion of the cell-to-cell distance (A) and the cell-to-substrate distance (h).

We investigated the effect of radiation exposure on the cell, using the ECIS method. The impedance of BAEC (Bovine Aortic Endothelial Cells) before and after the radiation exposure, the cell-to-cell and cell-to-substrate gaps, and micromechanical properties were evaluated. Impedance changes could be confirmed from just after the exposure. The result suggests that this method is very sensitive to the effect of radiation exposure on the cell, and this new methodology is expected to become a new technique in the field of the effect of radiation and electromagnetic waves.

2. Method

The electrical cell-substrate impedance sensing (ECIS) system method developed by Giaever et al. is a very sensitive electrical method for the detection of nanometer-order dynamics of cells cultured on a small gold electrode as shown in Fig. 1.

2.1. Equivalent Circuit and Modeling

The impedance of the electrode system can be considered as the equivalent circuit composed of the culture medium electrolyte impedance between cells in the direction perpendicular to the electrode (Z_{sol}), cell impedance (Z_c) and polarization impedance of the electrode (Z_p) as shown in Fig. 2 [4, 5].

Z_{sol} consists of resistance between cells in the culture medium (R_{sol}) and capacitance between cells in the culture medium (C_{sol}) and, in particular, R_{sol} is an important as the resistance component related to cell-to-cell distance (A). Z_c consists of capacitance of the cell membrane (C_c) and resistance of the cell membrane (R_c). Z_p has a constant angle and a magnitude, which depends on changes in f^{-m}, consisting of the equivalent series resistance component (R_{ps}) and the equivalent series capacitance component (C_{ps}), and X_{ps} is the reactance of C_{ps}. R_{bulk} is the bulk resistance.

The components of the equivalent circuit for the impedance of the electrode system were modeled as follows. Since the impedance of the culture medium between the cells (Z_{sol}), and the impedance of the cells (Z_c) can be approximated as a parallel circuit with R_{sol} and C_c, in this study, we proposed a more precise mathematical model for these components, that is, the Cole-Cole model with the distributed relaxation time, which is represented as follows:

a)

b)

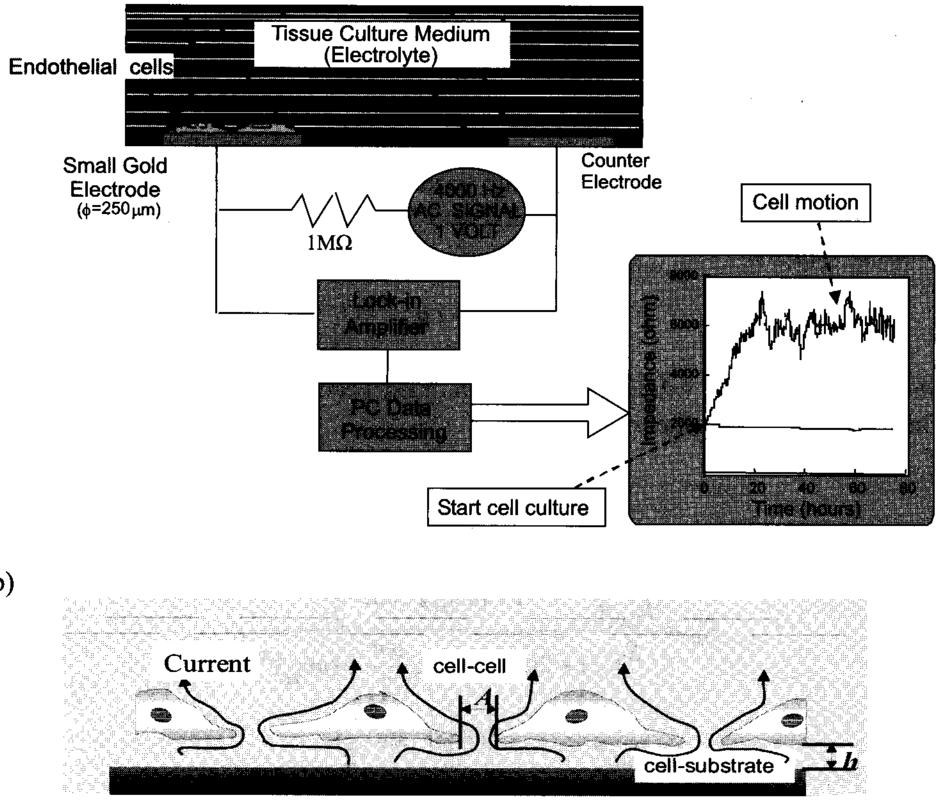

Fig. 1. Schematic diagram of ECIS system: (a) ECIS system, (b) cells on gold electrode (A and h)

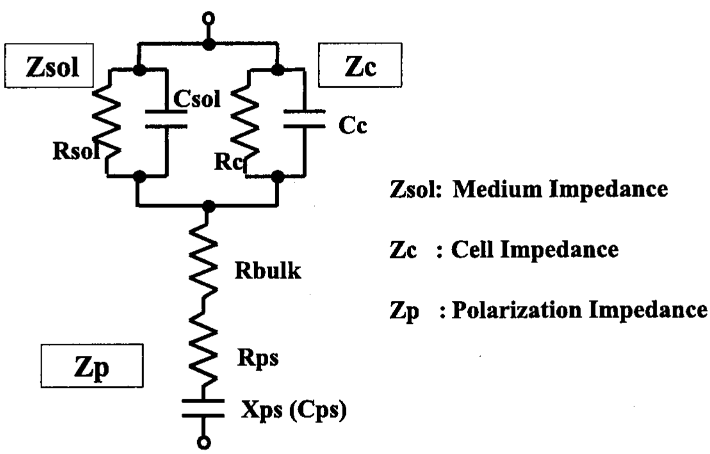

Fig. 2. Equivalent circuit of impedance for ECIS electrode system

$$Z_{cc} = Z_c \,//\, Z_{sol} = R_{st} + jX_{st} \\ = \frac{R_{sol}}{1+(j\omega\tau_m)^{\alpha}}, \tag{1}$$

where τ_m is the central relaxation time and α is a parameter for the distribution degree of the relaxation time, which is well known as Cole-Cole arc's law [6,7].
The cell-to-cell distance A directly influences R_{sol}. Then, new parameter S_A is introduced as follows:

$$S_A = R_{sol}^* / R_{sol}, \tag{2}$$

where R_{sol}^* is the R_{sol} value of standard (confluent) cell conditions.
Thus, $R_{sol} = R_{sol}^* / S_A$, therefore,

$$Z_{cc} = \frac{R_{sol}^*}{\{1+(j\omega\tau_m)^{\alpha}\} S_A}, \tag{3}$$

$$R_{st} = \frac{R_{sol}^*}{S_A} \frac{1+(\omega\tau_m)^{\alpha}\cos\dfrac{\alpha\pi}{2}}{1+2(\omega\tau_m)^{\alpha}\cos\dfrac{\alpha\pi}{2}+(\omega\tau_m)^{2\alpha}}, \tag{4}$$

$$X_{st} = \frac{R_{sol}^*}{S_A} \frac{(\omega\tau_m)^{\alpha}\sin\dfrac{\alpha\pi}{2}}{1+2(\omega\tau_m)^{\alpha}\cos\dfrac{\alpha\pi}{2}+(\omega\tau_m)^{2\alpha}}. \tag{5}$$

The polarization impedance of the electrode (Z_p) is expressed as the following equation:

$$Z_p = Z_0 f^{-m} e^{-j(\beta\pi)/2} \\ = R_{ps} + jX_{ps}, \tag{6}$$

where m denotes the power constant of f, and $\beta\pi/2$ is the phase angle of Z_p. Z_p strongly depends on the cell-to-substrate distance (h) because of the shielding effect of the cells on the electrode. Then, new parameter S_h is introduced as follows:

$$S_h = Z_0^* / Z_0, \tag{7}$$

where Z_0^* is the Z_0 value of standard (confluent) cell condition.
Thus $Z_0 = Z_0^* / S_h$, therefore,

$$Z_p = (Z_0^* / S_h) f^{-m} e^{-j(\beta\pi)/2}, \tag{8}$$

$$R_{ps} = (Z_0^* / S_h) f^{-m} \cos(\beta\pi)/2, \quad (9)$$
$$X_{ps} = (Z_0^* / S_h) f^{-m} \sin(\beta\pi)/2. \quad (10)$$

R_{bulk} is also expressed as follows:

$$R_{bulk} = S_h R_{bulk}^* \quad (11)$$

where R_{bulk} * is the bulk resistance under the normal condition (confluent) of the cell.

Then, the total impedance (Z_{total}) of the ECIS electrode system is as follows:

$$Z_{total} = R_{stt} + jX_{stt} \quad (12)$$
$$= R_{ps} + S_h^* R_{bulk} + R_{st} + j(X_{ps} + X_{st}). \quad (13)$$

Global parameters of the cultured confluent conditions of BAEC were determined as follows:

$\alpha = 0.778$, $\beta = 0.953$, $m = 1.321$, $\tau_m = 10.9\ \mu s$,

$R_{sol} = 8.85\ k\Omega$, $Z_0 = 0.841\ k\Omega$, $R_{bulk}^* = 1.10\ k\Omega$.

2.2. Parameters S_A, S_h and Evaluating A and h

An important function of the ECIS method is that the cell micro-dynamics, the cell-to-cell distance (A) and the cell-to-substrate distance (h) can be obtained from the impedance change in which two special parameters R_b and α are used as indicators of A and h [8]. In this paper, micro-motion of A and h can be analyzed easily by the vector impedance change based on the mathematical model of the ECIS system.

First, we compose a mathematical model for the standard (confluent) cell condition. Second, we make a lattice showing the impedance changes in cases of two parameter variations, S_A, S_h at each frequency. Third, we pile up the lattice over the vector impedance loci obtained by stimulation with X-ray or drug. We can obtain instantaneous values of S_A, S_h for the impedance change, that is, the changes of A and h. Although S_A, S_h and A, h are not a linear relationship, if A is decreased, S_A is decreased because R_{sol} is increased, and if h is decreased, S_h is decreased because Z_0 is increased.

2.3. Cell Culture in the ECIS System and the Radiation Exposure

Bovine aortic endothelial cells (BAEC) were purchased from Cell Systems (Kirkland, WA). BAEC were subcultured on a gelatin-coated cell culture dish (consisting of eight sections [10 mm×10 mm wide]; Applied Biophysics, Troy, NY) with a micro-electrode (250 μm dia.) and cultured with 5% CO_2 at 37°C until confluence. BAEC were then exposed to 100 Gy X-ray (150 kV). Electrical impedance was measured in an attachment mode (data are acquired every 30 seconds-1 min, impedance measurement frequency 4 kHz) and the frequency scan mode (impedances measured at the frequency from 25 Hz to 60 kHz) of ECIS.

3. Results

3.1. Comparison between Impedance of Cole-Cole Model and Cultured BAEC

The measurement results of the frequency characteristics of BAEC impedance at confluence are shown in Figs. 3 and 4. We determined the mathematical model in the form of the Cole-Cole model for the experimental results of BAEC. The results are also shown in Figs. 3 and 4. The vector impedance in Fig. 3 by the Cole-Cole model could be best fit to that of BAEC in the frequency range between 1 kHz to 10 kHz.

These frequency characteristics of the Cole-Cole model in Fig. 4 were almost the same as those of the experimental results of BAEC in the frequency range between 1 kHz to 10 kHz.

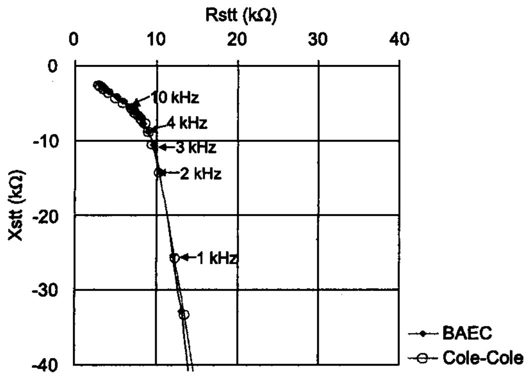

Fig. 3. Vector impedance loci of the Cole-Cole model and the cultured BAEC

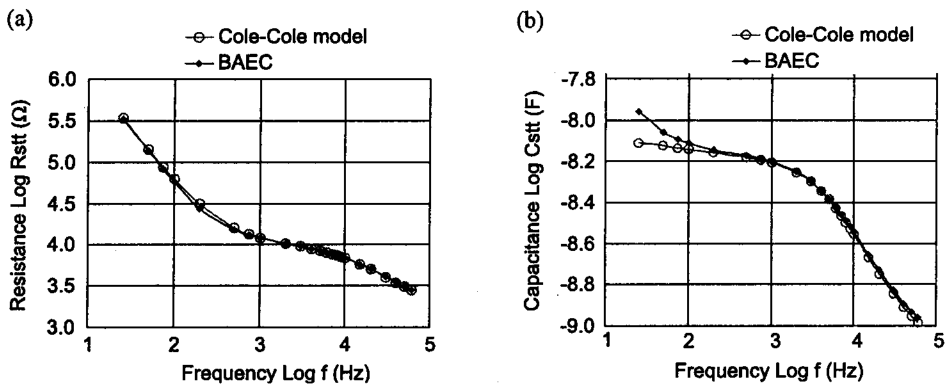

Fig. 4. Frequency characteristics from 25 Hz to 60 kHz of resistance and capacitance in the cultured BAEC and its Cole-Cole model: (a) equivalent series resistance, (b) equivalent series capacitance

3.2. Simulation of Variation S_A and S_h

From the mathematical model in Fig. 3, new vector impedance loci, which were determined by simulation of the model, are shown in Fig. 5. Each lattice shows the impedance changes in the case of two parameter variations S_A, S_h from 0.8 to 1.2 at frequencies of 1 kHz, 2 kHz, 4 kHz, and 10 kHz. The lattice forms depending on the changes of two parameters S_A, S_h are different in each frequency point. We can determine easily and instantly the parameter changes from the vector impedance using these lattices.

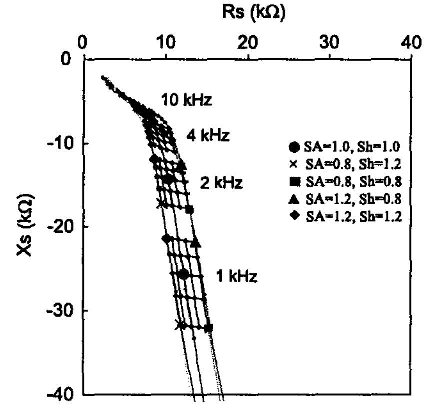

Fig. 5. Vector impedance loci of BAEC for different parameter values. Each lattice shows the impedance changes in case of two parameters variation S_A, S_h from 0.8 to 1.2 at the frequencies of 1 kHz, 2 kHz, 4 kHz and 10 kHz

3.3. X-ray-exposed Cultured BAEC

Vector impedance of the cultured cells for radiation exposure: BAEC cultured on the ECIS electrode were exposed to 100 Gy X-ray, and their impedances were measured. Fig. 6 shows six states of vector impedance loci measured in frequency scan mode: the result before the X-ray exposure and the results after the X-ray exposure.

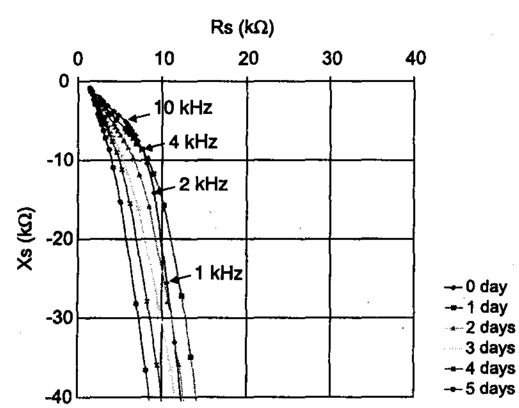

Fig. 6. Representative plotting of the variation of the vector impedance loci of cultured BAEC in every state before and after the X-ray exposure

The extended lattices covering the vector impedance loci with the X-ray exposure at 4 kHz and 2 kHz are shown in Figs.7 and 8, respectively. Based on these results, the impedance changes of the cultured BAEC after the X-ray exposure were analyzed, and the coefficients of S_A, S_h were determined at every impedance point. The results are shown in Fig. 9.

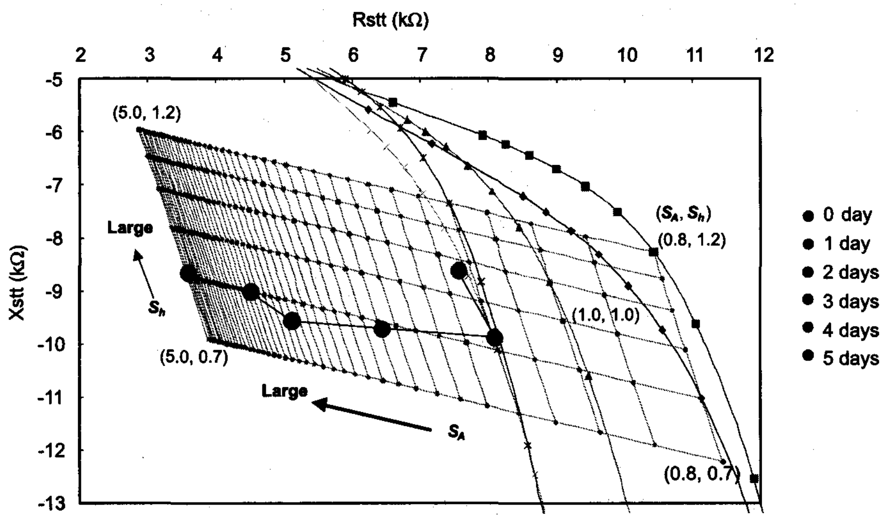

Fig. 7. Extended lattices covering the vector impedance loci at 4 kHz of BAEC with the 100 Gy X-ray exposure

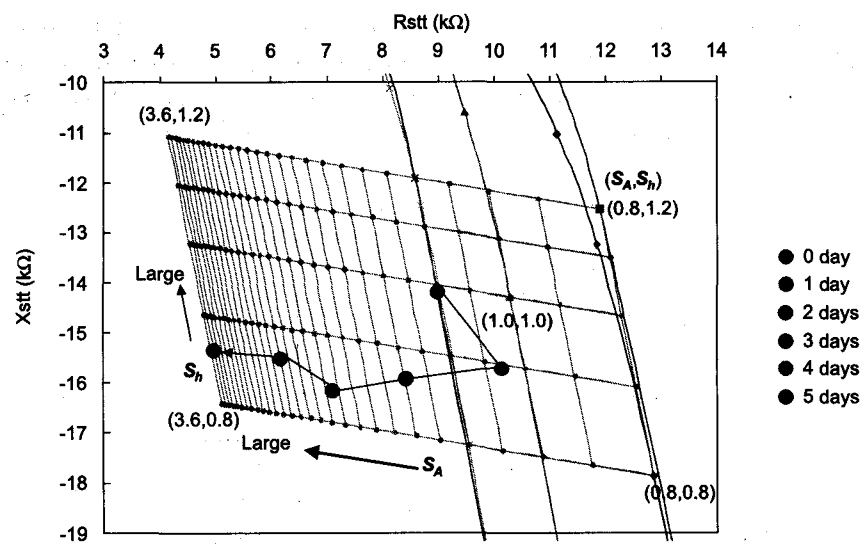

Fig. 8. Extended lattices covering the vector impedance loci at 2 kHz of BAEC with the 100 Gy X-ray exposure

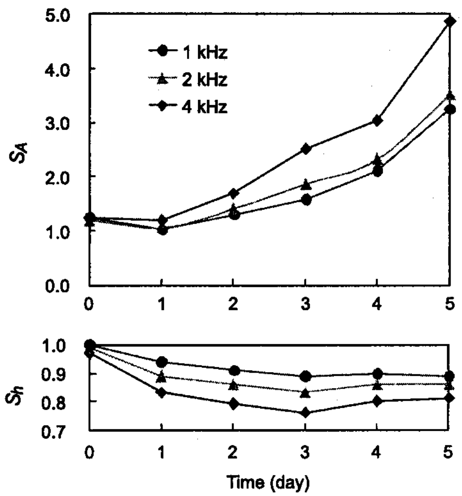

Fig. 9. Variations of S_A, S_h at 1 kHz, 2 kHz and 4 kHz after the X-ray expose on the cultured BAEC

After the exposure to 100 Gy X-ray, S_A increased gradually, and S_h decreased little in time at all the frequencies of 1, 2, 4 kHz. This means that A increased and h decreased. Under this condition, the decrease of the cell number could be confirmed by microscopic examination.

4. Discussion

4.1. Impedance Modeling of Cultured BAEC

We composed an equivalent circuit of impedance for the ECIS electrode system and a mathematical model for the electrical characteristics of the culture medium, cells and the electrode interface, according to measurements from the ECIS system. The mathematical model based on the Cole-Cole model was very much in agreement with the experimental values from 1 kHz to 10 kHz.

Furthermore, we introduced two parameters S_A, S_h for easy evaluation of the cell-to-cell distance (A) and the cell-to-substrate distance (h). Lattices composed of two parameter variations S_A, S_h can easily evaluate the changes of A and h. In general, an increase of A causes a decrease of resistance and the increase of h causes a decrease of the capacitive reactance.

4.2. Changes of Cultured BAEC with X-ray Exposure

As an application of this method, we tried to analyze the effect of radiation exposure of 1, 10, 100 Gy on BAEC. Impedance changes at 1 and 10 Gy could not be confirmed over a short time. It was proved that the 100 Gy X-ray exposure on BAEC resulted in a large decrease of the resistive component of impedance, that is, a large increase in the cell-to-cell distances (A), and a slight decrease in the cell-to-electrode distances (h), as shown in Fig. 9. There were some differences in S_A, S_h at every frequency, although the time trend was the same. There was no difference in A and h, respectively, at all the frequencies, the reason for this should be clarified.

This methodology is a real-time, continuous application without difficult handling, and it is multi-channel and can be expected to be used in the wide field of radiation and electromagnetic waves.

5. Conclusions

A mathematical model of impedance was proposed for micro-dynamics of cultured cells measured with ECIS. It was very much in agreement with the experimental values from 1 kHz to 10 kHz. Two parameters S_A and S_h were introduced for easy evaluation of the cell-to-cell distance (A) and the cell-to-substrate distance (h), respectively. The effect of the radiation exposure of 100 Gy was evaluated with parameters S_A and S_h. It was clarified that the cell-to-cell distance (A) increased significantly, and the cell-to-substrate distance (h) slightly decreased.

Acknowledgments

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