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(Original article)

Quantitative evaluation of micromotion of cultured cells using electrical cell-substrate impedance sensing (ECIS) method – cell-to-cell distance and cell-to-substrate distance –

Noriko GODA, Yoshitake YAMAMOTO, Takao NAKAMURA,
Toshimasa KUSUHARA, and Toshinori MARUYAMA

Summary

We have proposed a mathematical model for the micro-dynamics for cultured cells measured with ECIS system for the detection of nanometer-order dynamics of cells cultured on a small gold electrode and could separately evaluate cell-to-cell distance (A) and cell-to-substrate distance (h). For wide applications of this method, we constructed mathematical models which express cell-to-electrode impedances for some kinds of confluent conditions. Based on this mathematical model, we defined new parameters S_A and S_h in order to evaluate cell-to-cell distance and cell-to-substrate distance. As the application, we investigated the effect of X-irradiation to bovine aortic endothelial cell (BAEC). We analyzed the micro-dynamics of cells from the impedance of BAEC before and after X-irradiation. It was proved that the stimulation of 100 Gy X-irradiation to the BAEC resulted in the large scale of increase in the cell-to-cell distances (A), and the slight increase in the cell-to-substrate distances (h) accompany with continuous fluctuations.

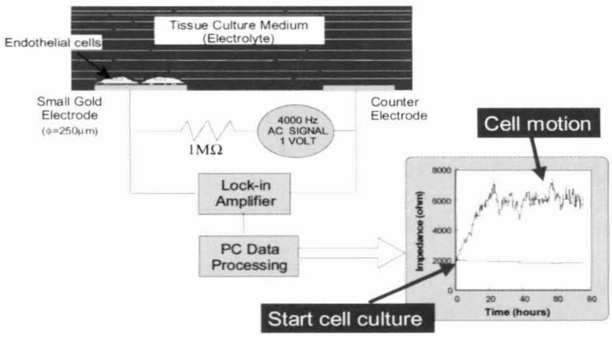
Key Words : ECIS method, bio-electrical impedance, cultured cell modeling, micromotion of cultured cells, X-irradiation

Introduction

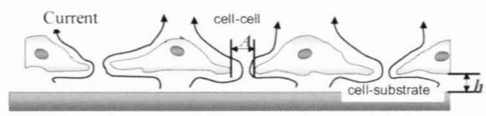
The electrical cell-substrate impedance sensing (ECIS) method has developed by Giaever et al. which is a very sensitive electrical method for the detection of nanometer-order dynamics of cells on a small gold electrode as shown in Fig. 1. We have proposed a mathematical model for the micro-dynamics for cultured cells measured with ECIS system that can separately evaluate cell-to-cell distance and cell-to-substrate distance[1-3]. For wide applications of this method, we constructed mathematical models describing for some kinds of cell and some kinds of confluent conditions. In this model, the polarization impedance of the electrolyte medium interface is constant phase angle and magnitude decreasing negative power function of frequency. The cell impedance, mainly cell membrane impedance is formed by the equation of Cole-Cole dispersion function in which system the relaxation time is continuously distributed over wide range.

In this study, new parameters S_A, S_h were introduced for evaluating a micro-motion of the cell-to-cell distance (A) and the cell-to-substrate distance (h). We investigated the effect of radiation exposure to the cell using ECIS method. We measured the impedance of bovine aortic endothelial cells (BAEC) before and after irradiation and evaluated the cell-to-cell and cell-to-substrate gaps and micromechanical properties. The impedance change could be confirmed from after X-irradiation. The result suggests that this method is very sensitive for the effect of X-irradiation on some cells and this new methodology is expected as new

Faculty of Health Sciences, Okayama University Medical School



(a) ECIS system



(b) cells on gold electrode (A and h)

Fig. 1 Schematic diagram of ECIS system.

technique in the field of the effect of radiation and electromagnetic waves.

Method

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 perpendicular direction to 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 the culture medium (R_{sol}) and capacitance between cells of the culture medium (C_{sol}), and in particular, R_{sol} is important as the resistance component related to the 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 the 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.

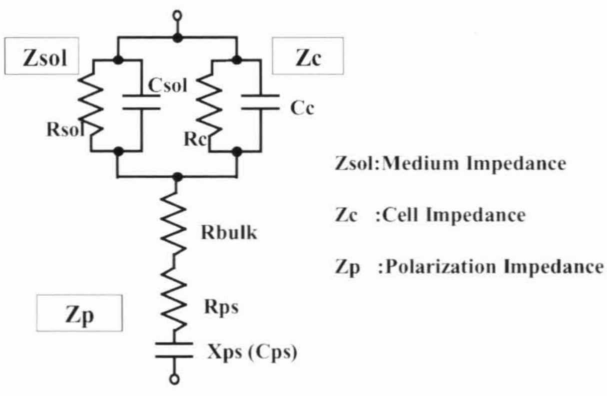


Fig. 2 Equivalent circuit of impedance for ECIS electrode system.

The components of the equivalent circuit for the impedance of the electrode system were modeled as follows. Since, the impedance of culture medium between cells (Z_{sol}), and the impedance of 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, Cole-Cole model with distributed relaxation time which is represented as follows:

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

Where τ_m is central relaxation time and α is parameter for distribution degree of relaxation time, which is well known as Cole-Cole arc's law [6,7].

The cell-to-cell distances A directly influences R_{sol}. Then, new parameter S_A is introduced as follows:

$$S_A = R_{sol}^{*} / R_{sol} \quad (2)$$

where R_{sol}^{*} is R_{sol} value of standard (confluent) conditions of cells.

Thus, $R_{sol} = R_{sol}^{*} / S_A$, therefore,

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

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

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

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

$$\begin{aligned} Z_p &= Z_o f^{-m} e^{-j(\beta\pi)/2} \\ &= R_{ps} + jX_{ps} \end{aligned} \tag{6}$$

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 cells on the electrode. Then, new parameter S_h is introduced as follows:

$$S_h = Z_o^* / Z_o \tag{7}$$

where Z_o^* is Z_o value of standard (confluent) all condition of cells.

Thus $Z_o = Z_o^* / S_h$, therefore,

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

$$R_{ps} = (Z_o^* / S_h) f^{-m} \cos(\beta\pi)/2 \tag{9}$$

$$X_{ps} = (Z_o^* / S_h) f^{-m} \sin(\beta\pi)/2 \tag{10}$$

R_{bulk} is also expressed as follows:

$$R_{bulk} = S_h R_{bulk}^* \tag{11}$$

where R_{bulk}^* is bulk resistance under the normal condition (confluent) of cell. Then, the total impedance (Z_{total}) of ECIS electrode system is as follows:

$$Z_{total} = R_{stt} + jX_{stt} \tag{12}$$

$$= R_{ps} + S_h^* R_{bulk} + R_{st} + j(X_{ps} + X_{st}) \tag{13}$$

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

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

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

2) Parameters S_A, S_h and evaluating A and h

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

First, compose a mathematical model for standard (confluent) cell condition. Second, make lattice shows the impedance changes in some cases of two parameters variation S_A, S_h at each frequency. Third, pile up the lattice over the vector impedance loci obtained by some stimulation with X-ray or drag. 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 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_o is increased.

3) Cell culture in the ECIS system and irradiation treatments

The BAEC were purchased from Cell Systems (Kirkland, WA), it is easy to culture in mono layer. The BAEC were subcultured on a gelatin-coated cell culture dish (consisting of eight sections [10mm × 10mm wide]; Applied Biophysics, Troy, NY) with a microelectrode (250μm dia.) and incubated for 48hr at 37℃ in a 5% CO_2 / 95% air atmosphere until confluence. The BAEC were then irradiated at a single dose of 100Gy by X-ray (maximum rated output voltage: 150kV) from an X-ray generator MBR-1505R2 (Hitachi Co., Ltd.). Electrical impedance was measured with attachment mode (the data are acquired every 30 to 60seconds, impedance measurement frequency 4 kHz) and frequency scan mode (impedances measured at the frequency from 25Hz to 60kHz) of ECIS.

Results

1) Comparison between Impedance of Cole-Cole model and cultured BAEC

The measurement results of frequency characteristics of BAEC impedance at confluent condition are shown in Fig. 3 and Fig. 4. We determined the mathematical model in form of Cole-Cole model for the experimental results of BAEC. The results are also shown in Fig. 3 and Fig. 4. Vector impedance in Fig. 3 by Cole-Cole model could be best fit

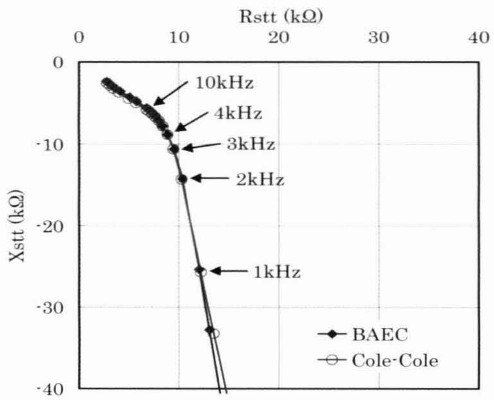
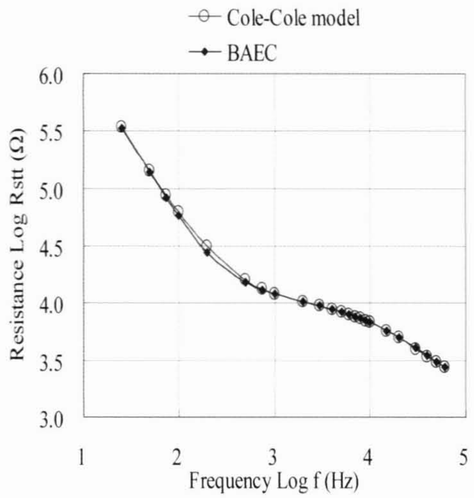
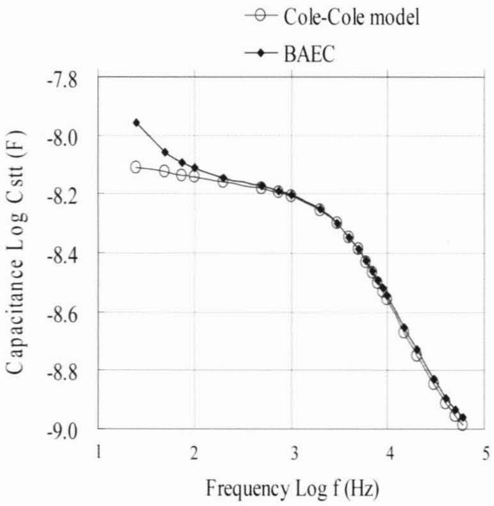


Fig. 3 Vector impedance loci of Cole-Cole model and cultured BAEC.



(a) equivalent series resistance



(b) equivalent series capacitance

Fig. 4 Frequency characteristics from 25Hz to 60kHz of resistance and capacitance in the cultured BAEC and its Cole-Cole model.

to that BAEC in the frequency range between 1 kHz to 10kHz.

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

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 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 10kHz. 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 parameter changes from vector impedance using these lattices.

3) Changes of cultured BAEC by X-irradiation

Vector impedance of cultured cells for irradiation: BAEC cultured on the ECIS electrode were irradiated at a dose of 100Gy and their impedan-

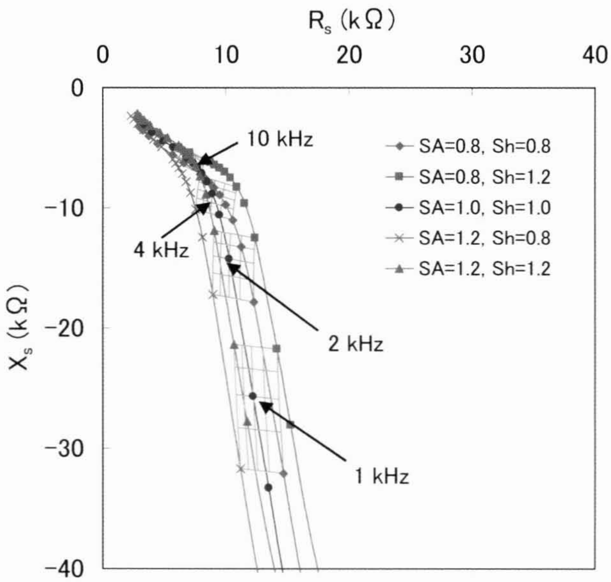


Fig. 5 Vector impedance loci of BAEC for different parameter values. Each lattice shows the impedance changes in case of two parameters variation S_A and S_h from 0.8 to 1.2 at frequencies of 1 kHz, 2 kHz, 4 kHz, and 10kHz.

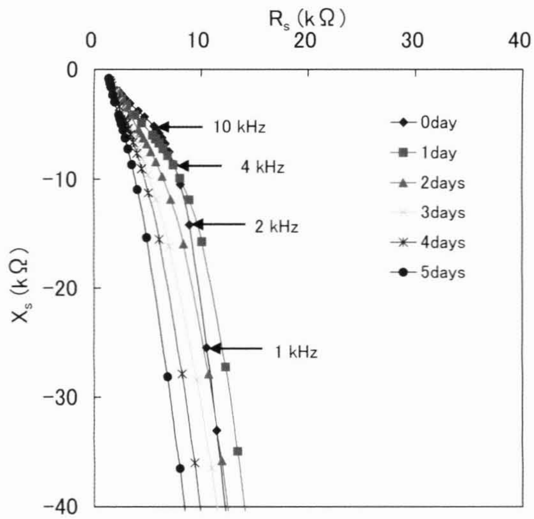


Fig. 6 Representative plotting of variation of vector impedance loci of cultured BAEC at the every state before and after X-irradiation. (0day : before of X-irradiation)

ces were measured. Fig. 6 shows six states of vector impedance loci measured in frequency scan mode: the result before X-ray expose and the results after X-irradiation.

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

After 100Gy X-irradiation, S_A increased gradually and Sh decreased little overtime day at all frequencies of 1, 2 and 4 kHz. This means that A increased and h decreased. Under this condition, decrease of cell number could be confirmed by microscopic examination.

Discussion

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

Furthermore, we introduced the two parameters

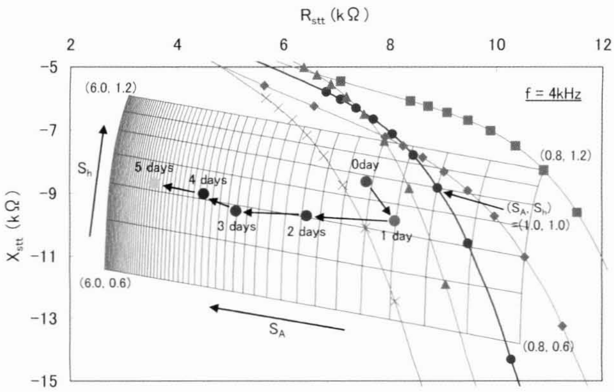


Fig. 7 Extended lattices covering vector impedance loci at 4 kHz of BAEC with 100Gy X-irradiation.

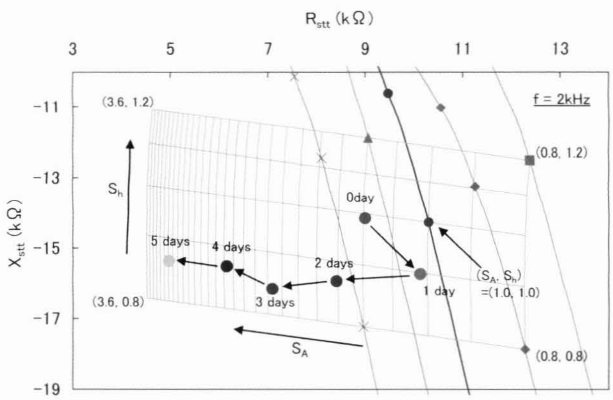


Fig. 8 Extended lattices covering vector impedance loci at 2 kHz of BAEC with 100Gy X-irradiation.

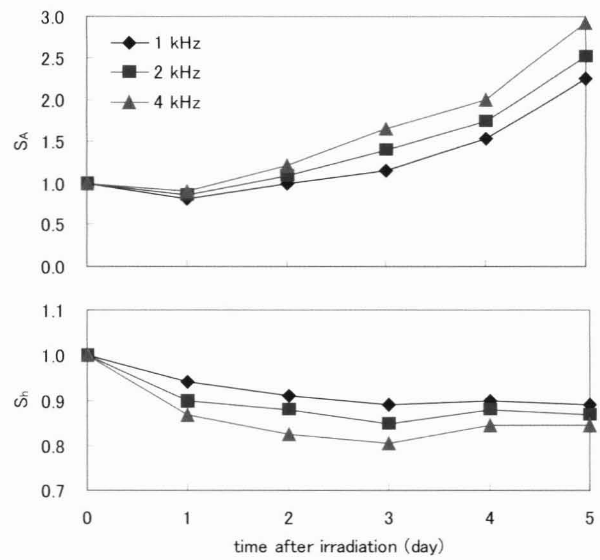


Fig. 9 Variations of S_A and S_h at 1 kHz, 2 kHz and 4 kHz after X-irradiation to cultured BAEC. Each value of S_A and S_h has been rearranged by the value of 0 day before X-irradiation.

S_A, S_h for easy evaluation of the cell-to-cell distance (A) and the cell-to-substrate distance (h). This research copes with the method of Giaever[1,2] who use R_b, α in the point making the same electrode model a base. Therefore, the principle of this research is trustworthy in the same way as Giaevers' research. The characteristics of this research are that it can be analyzed easily by using the limited information. Lattices composed of two parameters variation S_A, S_h can easily evaluate the changes of A and h. In general, the increase of A causes decrease of resistance and the increase of h causes decrease of capacitive reactance.

As the application of this method, we tried to analyze of the effect of irradiation of 1, 10, 100Gy on the BAEC. Impedance changes at 1 and 10Gy could not be confirmed over a short time. It was proved that 100Gy of X-irradiation on the BAEC resulted in a large decrease of resistive component of impedance, that is, a large increase in cell-to-cell distances (A), and a slight decrease in the cell-to-substrate distances (h) as shown in Fig. 9.

Usually, the influence of the X-irradiation toward the cultivation cell is evaluated at the cell existence rate after the irradiation, and it is greatly different from this research. Therefore, comparison is difficult with this research result and other.

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 frequency, the reason for this should clarified. Furthermore, determination of dimension of A and h themselves is future subject.

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

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（原　著）

Electrical Cell-substrate Impedance Sensing (ECIS) 法を用いた培養細胞の微細挙動の定量的評価法　－細胞－細胞間隙と細胞－電極間隙の評価－

合田典子，山本尚武，中村隆夫，楠原俊昌，丸山敏則

抄　　録

［背景］Electrical Cell-substrate Impedance Sensing（ECIS）は培養細胞の電気的計測により，その微細挙動を評価する工学的手法である。我々はECISを用いてこの微細挙動を細胞-細胞間隙と細胞-電極間隙に分離して推定することが可能な数学的モデルを提案してきた。本研究ではこの数学的モデルを使用して，X線を照射した牛大動脈内皮細胞（bovine aortic endothelial cell：BAEC）の微細挙動の経時変化を評価した。

［方法と結果］ECISシステム（Model 1600R Applied BioPhysics）を用いてBAECのコンフルエント到達前後で計測を行い，数学的モデルを構築した。このモデルは細胞の微細挙動を検出するために重要な周波数レンジである1-10 kHzにおいて測定結果とよく一致し，Cole-Cole円弧則に従う。さらに細胞-細胞間距離Aの増減に対応する校正定数S_Aと細胞-電極間距離hの増減に対応する校正定数S_hを導入し，ベクトルインピーダンスの変化に対応した値を算出することで細胞の微細挙動を評価することとした。次に本法によりX線（150 kV，100 Gy）を照射したBAECの微細挙動を評価した。X線照射細胞では時間経過と共に抵抗成分の変化が支配的なインピーダンスの減少が確認された。この現象はS_Aの大きな増加とS_hの微小な減少をもたらした。このパラメータの変化は細胞間隙が拡大したことを示しており，X線照射による細胞内損傷により細胞密度が低下したと考えられた。

［結論］本法は培養細胞の微細動態の変化を細胞－細胞間隙と細胞－電極間隙に分けてリアルタイムに定量評価することが可能であり，各臓器の細胞レベルでの薬物の治療効果やX線に対する耐性の評価に適応できるものと考える。

キーワード：ECIS法，生体インピーダンス，細胞培養，細胞微細挙動，放射線被曝

岡山大学医学部保健学科