MICRO HOLE BASED SINGLE CHIP

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Abstract: Research with single cell chip enables to observe the condition or behaviour of single cell under specific environments, and to reduce the amount of samples required for experiments. Using impedance spectroscopy, the single cell can be characterised non-invasively and without chemical markers. In this paper, the current density and impedance of the micro hole-based cell chip during the cultivation were simulated by the finite element method (FEM). From the experiments, it was shown that the impedance of single cell can be measured sensitively enough to determine the cell position and growth, and the integrity of cell membranes. Further, an application of the micro hole based single cell chip as biosensors was evaluated from a toxic test.

Introduction

Utilization of cell based systems increases in cell and tissue engineering, for instance, to develop new medicaments and therapies against different diseases. Research with single cell chip enables to observe the condition or behaviour of single cell under specific environments, and to reduce the amount of samples required for experiments. Advances of the micro electro mechanical system (MEMS) make it possible to handle, separate, select, and position even the single cell. Using impedance spectroscopy, the single cell can be characterised non-invasively and without chemical markers. Since cell structures and physiological events determine the electrical properties of cells, the single cell can be monitored quantitatively by impedance spectroscopy [1].

For the research with single cell chip, we have developed a micro hole based chip consisting of a SiO_2 culture well, an insulated Si_3N_4 membrane (thickness of 800 nm) including the micro hole (diameter of several μm), and a micro fluidic system [2]. By properly controlling the micro fluid, a single cell is positioned on the micro holes along the fluidic flow without damage. During the impedance measurements, a low frequency current should be concentrated near the micro hole due to the insulated membrane and affected by the conditions of cell on the hole. However, in the enough high frequency range, the current can penetrate through the insulated membrane or leaked by stray capacitances. These expected physical phenomena should be validated by theoretical and experimental results. By exactly understanding the influence of the physical phenomena on the measured impedance, the boundary about the utilization and application of the micro hole based single cell chip can be clearly defined.

For the theoretical approach, in this paper, the electrical characteristics of the micro hole based cell chip were simulated by the finite element method (FEM). For the experimental approach, the impedances of the micro hole based cell chip were measured during the cultivation. Further, an application of the micro hole based single cell chip as biosensors was evaluated from a toxic test.

Materials and Methods

Theretical Approach: If the cell and cell culture medium are homogenous, source-free regions and linear volume dielectric in the electrical frequency range of 200 Hz to 1 MHz, and if the magnetic fields in them are negligible, then the potential distribution induced by current sources satisfies the generalized Laplace's equation. Further, it was assumed that the electrical properties of cell, cell culture medium, and Si_3N_4 membrane are constant in this frequency range. The electrical properties of the cell, cell culture medium and insulated membrane were shown in Table 1. The sensitivity field can be obtained by using volume dielectric analysis involving the lead field theory and the reciprocal energization of leads [3]. The total measured impedance Z considering the sensitivity field is as following.

$$Z = \int_V \frac{\vec{J}_1 \cdot \vec{J}_2}{(\sigma + i\omega\varepsilon) I^2} dv \qquad (1)$$

where σ and ε are the conductivity and permittivity, respectively, $i = (-1)^{1/2}$, $\omega = 2\pi f$ with f the frequency of the electric field, $\vec{J}_1$ the current density when a current I flows between the two current electrodes, and $\vec{J}_2$ the current density when I flows between the two voltage electrodes.

For the simulation, we designed a symmetrical cylinder model with a single cell and an insulated membrane. When the cell is positioned on the hole by the suction with a proper level, it was assumed that none part of the cell is inserted into the hole, and that the shape of cell is a half spheroid. The symmetrical cell was positioned at the middle of hole. The contact area of culture medium on the insulated membrane was 0.732 mm^2. The thickness of cell membrane was 10 nm, and the polar radius of half spheroid cell was 5 μm. The

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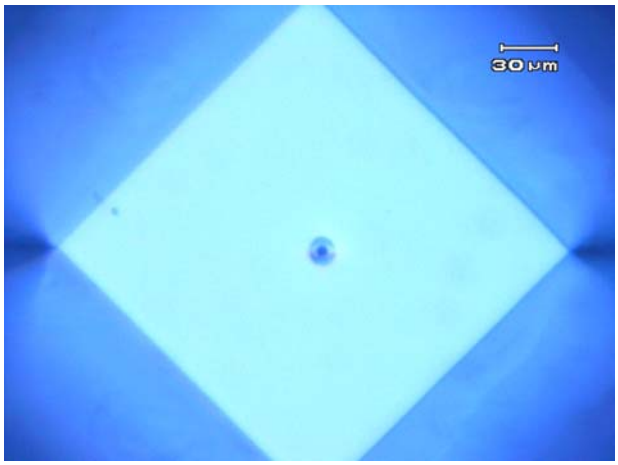
Table 1: Electrical properties of materials used for FEM simulation [1,3,5]

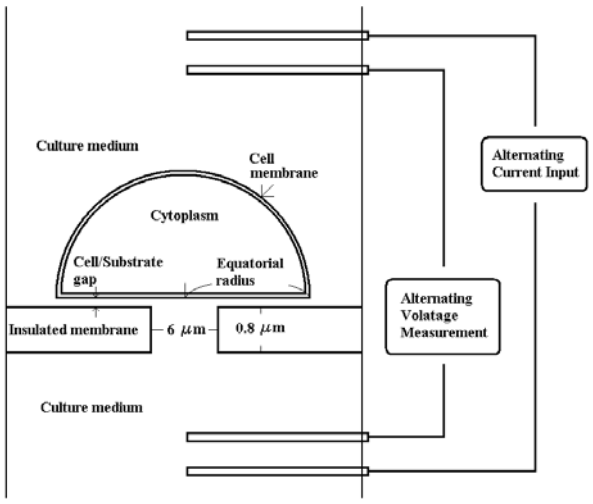
Materials	Conductivity (S/m)	Relative permittivity
Cytoplasm	0.5	80
Cell membrane	10^{-7}	11.3
Culture medium	1.5	80
Si_3N_4 membrane	10^{-12}	5.5

input current was 1 μA with the frequency range of 200 Hz ~ 1 MHz. With different cell/substrate gap and equatorial radius of cell, the current density and impedance were simulated in a symmetrical cylinder model by FEM (used software FlexPDE, PDE solutions).

Experimental Approach: For the experiment, a L929 cell in the cell culture medium (89.5 % RPMI 1640, 10 % FKS, 0.5 % Penicillin / Streptovidin) was positioned on one micro hole in one second by the micro fluidic controller. Using an impedance analyzer (Solartron 1260), the impedance of the cell chip was measured after positioning the cell and after culturing the cell on the hole for two days (under 7.5 % CO_2, 37 ºC) at the room temperature. Figure 1 shows a single cell positioned on one micro hole (Figure 1a) and the schematic of impedance measurement with micro hole based cell chip (Figure 1b). The peak and the frequency range of input potential were 10 mV and 1 Hz to 1 MHz, respectively. For the toxic test with this cell chip, 1 nl cell culture medium with 5% dimethlysulfoxide (5% DMSO) was prepared. After applying "5% DMSO" to the single L929 cell chip, the impedance of cell chip was measured at 1 kHz according to time.



(a)



(b)

Figure 1: (a) A L929 cell positioned on the micro hole, and (b) the schematic of impedance measurement with micro hole-based cell chip.

Results

At different frequencies, simulated current densities near a single cell positioned on one hole (equatorial radius of cell: 5 μm, cell/substrate gap: 150 nm) are shown in Figure 2. The red and violet coloured regions indicate the highest and the lowest value of current density, respectively. At 10 kHz (Figure 2a), the current is blocked by not only the Si_3N_4 membrane but also the cell membrane due to its high resistivity. Therefore, the low frequency current flows in the cell/substrate gap and results in the high magnitude of current density. However, it is observed that the current with the high frequency of 100 kHz (Figure 2b) or 1 MHz (Figure 2c) can penetrate the cell.

Figure 3 shows the simulated impedance magnitude of micro hole based chip with different cell/substrate gap (Figure 3a) and equatorial radius of cell (Figure 3b) versus the log scaled frequency. In case of "No cell", the impedance magnitude in the low frequency range (Z_{lowf}) is equal to the sum of the spreading resistance $(1/(4r_h\sigma))$ from the hole and the resistance inside hole.

$$Z_{lowf} = 2/(4r_h\sigma) + h_m/(\sigma\pi r_h^2) \approx 130\ \text{k}\Omega \qquad (2)$$

where, r_h and h_m are the radius and thickness of hole, respectively.

If the equatorial radius of cell increases and if the cell/substrate gap decreases, the impedance magnitude of cell chip increases in the low frequency range. However, the impedance magnitude decreases due to the stray capacitance when the frequency is above than about 3 kHz.

Measured impedance magnitude and phase of the cell culture medium (No cell), after positioning of the single L929 cell on the hole (L929 after positioning), and after cultivation of the cell for two days (L929 cultured for 2 days) are shown in Figure 4. The impedance magnitude of "No cell" is about 140 kΩ in the low frequency range. In case of "L929 cultured for 2 days", the uneven impedance magnitude is observed in the low frequency range. At 1 kHz, the impedance

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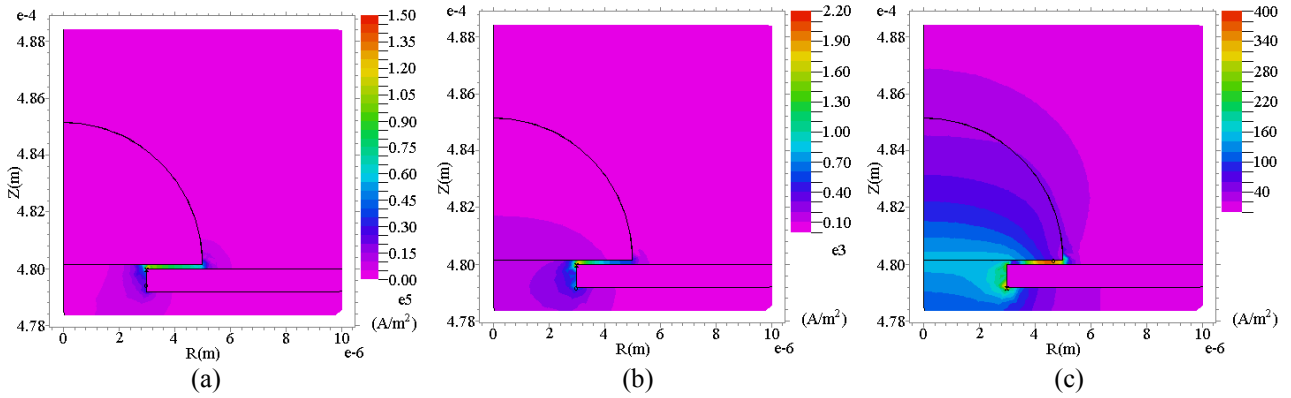

Figure 2: Simulated current densities near a single cell positioned on one hole (equatorial radius of cell: 5 µm, cell/substrate gap: 150 nm) at (a) 10 kHz, (b) 100 kHz, or (c) 1 MHz. The input current is 1 µA (used software FlexPDE, PDE solutions).

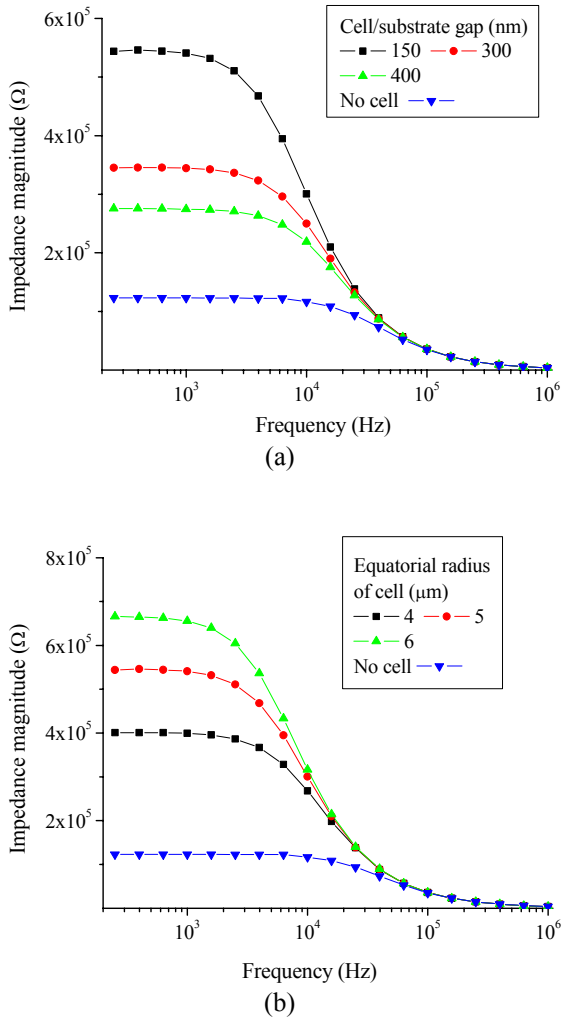

Figure 3: Impedance magnitude with (a) different cell/substrate gap (equatorial radius of cell: 5 µm) and (b) equatorial radius of cell (cell/substrate gap: 150nm) vs. the log scaled frequency, simulated by FEM (used software FlexPDE, PDE solutions).

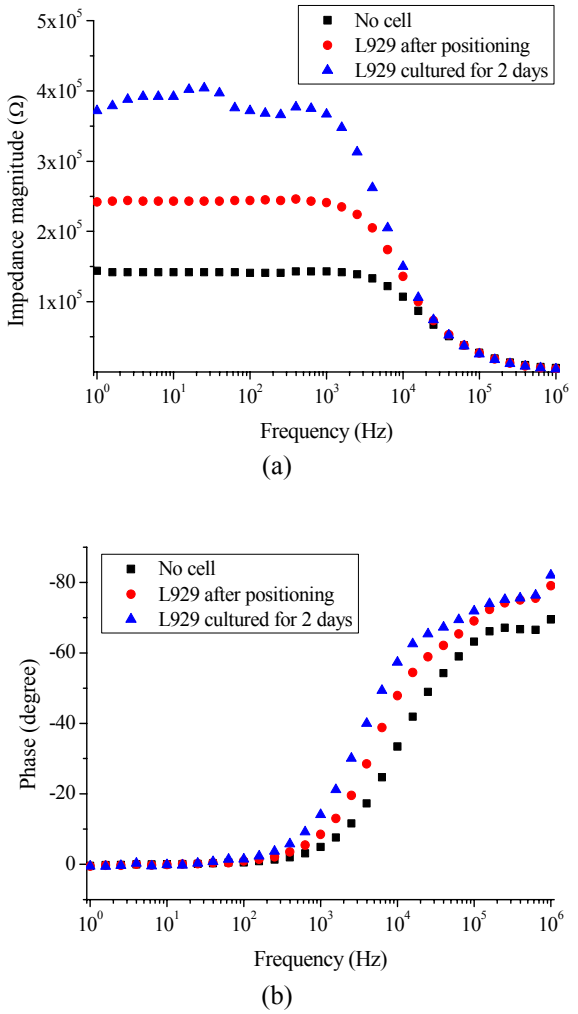

Figure 4: (a) Impedance magnitude and (b) phase of the cell culture medium (No Cell), the cell after positioning on the hole (L929 after positioning), and the cell after cultivation for two days (L929 cultured for 2 days) vs. the log scaled frequency, measured by Solartron 1260 from 1 Hz to 1 MHz at the room temperature. The peak of the input signal is 10 mV.

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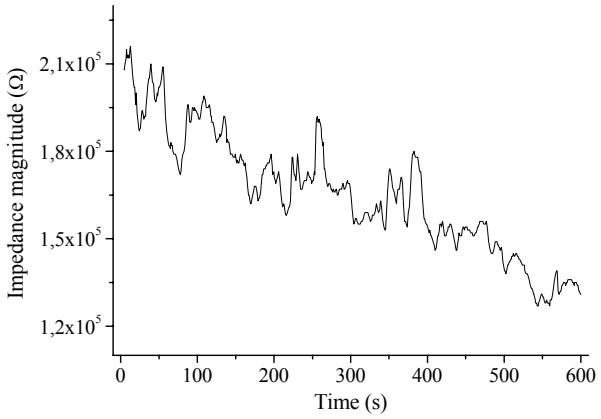
magnitude of "L929 after positioning" is measured higher with the value of 97.8 kΩ than "No cell". Also, the impedance magnitude in "L929 cultured for 2 days" is more increased with the value of 125.49 kΩ than "L929 after positioning" at 1 kHz. However, these differences of impedance magnitudes decrease as the increase of frequency, and disappear above about 10 kHz.

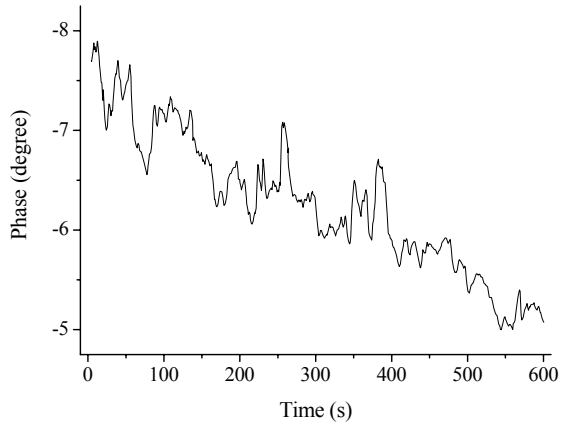
Figure 5 shows the result about the toxic test with the micro hole based single L929 cell chip using impedance spectroscopy. After positioning a L929 cell on the hole, the impedance magnitude and phase measured at 1 kHz and the room temperature are about 241 kΩ and – 8.47 °, respectively. After applying the "5% DMSO" to the cell chip, the impedance magnitude is decreased and the phase is increased gradually at 1 kHz according to time. The cell membrane is destroyed by 1 nl "5% DMSO".

Discussion

Micro hole-based chip can be utilized for fast positioning cells on the required locations by controlling micro fluidic. With the four-electrode system, the micro hole-based chip can avoid the effect of electrode polarisation and the shadowing effect occurred when the currents are canalised with uneven current density near the sample on the electrode [1]. Therefore, this micro hole-based cell chip can be used to measure the impedance of sample accurately in the low frequency range. However, the enough high frequency current can penetrate the Si_3N_4 membrane also. Therefore, to design the micro hole-based cell chip for a biosensor using impedance spectroscopy, it is required to understand the electrical characteristics near the cell on the hole.



(a)



(b)

Figure 5: After applying the "5% DMSO" to the single L929 cell chip, (a) the impedance magnitude and (b) phase measured at 1 kHz and the room temperature according to time.

From the simulations and measurements, it is shown that the low frequency current is blocked by the insulated membrane and the cell membrane with high resistivity. Therefore, the micro hole-based cell chip has the high sensitivity about the cell conditions on the hole in the low frequency range, because the current flows through the hole and the cell/substrate gap. When positioning the L929 cell on the micro holes, the suction level must be controlled carefully not to damage the cell. The well positioned cells is ensured by the increase of impedance magnitude in the low frequency range (< ~ 3 kHz) than a control. As the cell grows, the cell more spreads and adheres to the substrate [4]. From the Figure 3 and Figure 4, it is theoretically and experimentally proved that the micro hole-based cell chip can be used to monitor the cell growth using impedance spectroscopy with high sensitivity. With higher frequencies, the current can penetrate the cell membrane and cytoplasm (see Figure 2). In the enough high frequency range, however, the current flow also through the Si_3N_4 membrane. The dimension of hole such as the radius or thickness of hole determines the electrical characteristic of micro hole-based chip. By changing the hole dimension or the contact area of culture medium on the insulated membrane, the frequency beginning to show the stray capacitive characteristic can be controlled. From the toxic test, it is found that this micro hole-based single cell chip can be utilized for sensing toxic materials (see Figure 5).

Using impedance spectroscopy, the condition of the single cell positioned on the micro hole can be monitored quantitatively. It is expected that the automated micro hole based sigle cell chip using impedance spectroscopy will be broadly utilized in cell engineering.

Conclusions

Using FEM simulation, the current density near a single cell positioned on the micro hole and the impedance of the micro hole-based single cell chip were simulated. During the cultivation, the impedance of micro hole-based single L929 cell chip was measured by the four-terminal electrode arrangement. Further, with impedance spectroscopy, the micro hole based single L929 cell chip was used for sensing the toxic materials. This micro hole based single cell chip has the potential for monitoring the growth and the membrane integrity of single cell without any labelling.

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