IN VITRO MONITORING OF L929 CELLS GROWTH USING IMPEDANCE SPECTROSCOPY

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Abstract: Cell-based micro systems support the development of new medicaments and therapies against different diseases. Recently, numerous screening systems using impedance spectroscopy have been developed for *in vitro* monitoring of cells growth or behaviour. For the correct interpretation of the impedance change related to the condition of cells on electrodes, it is required to understand the reasons which lead to the change of electrical characteristic of the cell-electrode system. In this study, the growth of L929 cells was investigated. By fitting the equivalent circuit to the measured data during the cultivation, the quantitative analysis of the cell growth on the electrode was shown.

Introduction

Cell-based test systems can support the development of new medicaments and therapies against different diseases. Further, bio-monitoring of toxic materials in environment or foods is possible. Recently, numerous screening systems using impedance spectroscopy have been developed for *in vitro* monitoring of cells growth or behaviour. Using the impedance spectroscopy, the micro-motion of cells on an electrode and the behaviour of cell layers to the toxic heavy metal ions were monitored [1-3]. We have also developed a monitoring system consisting of a culture dish and a planar electrode structure with electrical interface, and measured the impedance of tumour spheroids including the toxic test [4].

For the correct interpretation of the impedance change related to the condition of cells on electrodes, it is required to understand the reasons which lead to the change of electrical characteristic of the cell-electrode system. Advances in micro electro mechanical system (MEMS) enable to reduce the size of electrode structures and the amount of samples. However, with decreasing the size of electrodes, the sensitivity of impedance related to the condition of cells on the total measured impedance decreases in the low frequency range because of the high impedance of electrode polarisation. On the other hand, at high frequencies, the measurements are influenced by leakage currents caused by stray capacitances of the system. Therefore, the electrode configuration of cell chip and measurement system should be designed properly for the *in vitro* monitoring of cell conditions.

In this study, the growth of L929 cells was investigated by using the electrode based cell chip and impedance measurement system. By fitting the equivalent circuit to the measured data during the cultivation, the quantitative analysis of the cell growth on the electrode was shown. From the results, the prospects of electrode based cell chip was discussed.

Materials and Methods

Cell chip, Measurement system and Experiments: By semiconductor technology, circular gold electrodes (sensing electrode) and lines were patterned on the silicon nitride layer deposited onto the silicon substrate. For the insulation of transmission lines, the silicon nitride was again deposited on the whole substrate by plasma-enhanced chemical vapour deposition. Then, the sensing electrodes and the terminals of lines were opened by reactive ion etching. The radius of exposed sensing electrode was 240 μm. The experimental setup was shown in Figure 1. The opened terminals of lines were connected with external transmission cables of an impedance analyzer. To culture the cells on the sensing electrode, a plastic dish conserving the cell culture medium was integrated with the electrode structure. For the counter electrode, a large platinum electrode was configured enough far above the sensing electrode. Further, a microscope for observing the cells growth on the sensing electrode was prepared.

For the experiments, the impedance of only culture medium (89.5 % RPMI 1640, 10 % FKS, 0.5 % Penicillin / Streptovidin) was first measured from 100 Hz to 1 MHz at 37 ºC by the impedance analyzer (Solartron 1260). The peak of input potential was 50 mV. After dropping the L929 cells (3.6×10^6 / ml) into the cell culture medium on the electrode structure, the

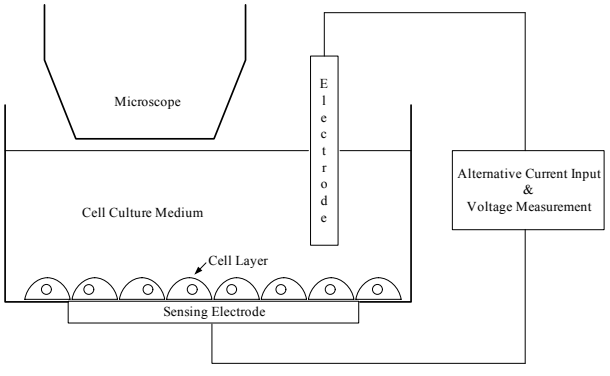

Figure 1: Measurement setup for *in vitro* impedance monitoring of cells.

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impedance was measured. Then, the impedance measurement was repeated after culturing the cells on the electrode structure in an incubator (Heraeus BB 6220) for 3 hours, 6 hours, 9 hours, 1 days, 2 days, and 3 days at 7.5 % CO_2 and 37 ºC. The culture medium on the electrode structure was refreshed every day during the cultivation. Further, the growth of L929 cells on the sensing electrode was observed by the microscope.

Equivalent circuit modelling and Fitting: Figure 2(a) shows the equivalent circuit in case of no cell on the electrode. The interfacial impedance between the electrode and electrolyte can be described by a constant phase element CPE_{el} $(1/T(i\omega)^P)$ for frequencies > 1 Hz [5]. Here, T and P are parameters of the constant phase element. C_{stray} is the parasitic stray capacitance. The spreading resistance R_{sol} from a circular electrode with the radius of r is $1/(4r\sigma)$ [6]. Figure 2(b) shows an equivalent circuit in case of cells on the electrode. R_{cl} and C_{cl} indicate the resistance and the capacitance of cell layer, respectively. R_{med} is the resistance of medium. The equivalent circuits in Figure 2 were fitted to the measured impedance by using a software (Scribner Associates ZView).

Results

During the cultivation, the measured impedances versus the log-scaled frequency are shown in Figure 3(a). In the low frequency range (< ~ 3 kHz), the impedances show mainly the characteristic of CPE_{el}, and thus the impedance magnitudes decrease gradually according to the increase of frequency. From 10 kHz to 300 kHz, the impedance magnitudes become almost constant, and increases according to the cultivation period. In case of no cells, the impedance magnitude measured at the frequency range of 10 kHz to 300 kHz is equal to the spreading resistance from the sensing electrode. When the frequency is above 300 kHz, the impedance magnitudes decreases because of the stray capacitance C_{stray}. From the normalized impedance change $(Z_{cell} - Z_{No\ cell})/ Z_{No\ cell}$ in Figure 3(b), it is found that the impedance measurement at nearly 40 kHz is most sensitive to monitor the cell growth with this system. From the pictures of L929 cells on the sensing electrode in Figure 4, it is shown that the cells adhere and proliferate well onto the sensing electrode during the cultivation. More densely populated cells on the sensing electrode cause higher impedance magnitude.

Figure 5(a) shows the fitting the equivalent circuits in Figure 2 to measured impedances when no cells on the sensing electrode (No cells) or after culturing for 3 days (3 days). From Figure 5(b), it is shown that the fitted R_{cl} in the equivalent circuit of Figure 2(b) increases but the fitted C_{cl} decreases according to the increase of cultivation period.

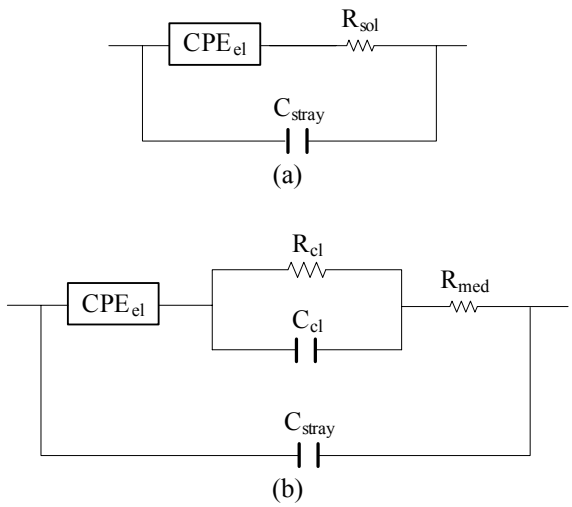
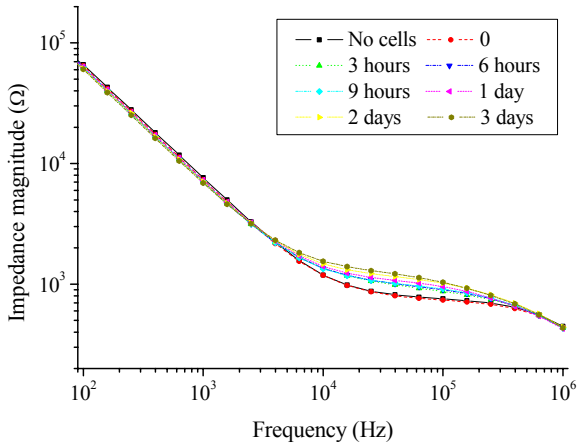
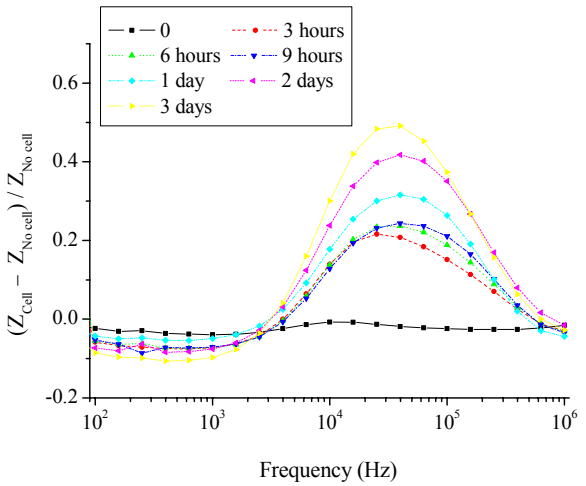


Figure 2: Equivalent circuit in case of (a) no cells or (b) cells on the electrode.



(a)



(b)

Figure 3: During L929 cultivation, (a) measured impedance magnitude and (b) normalized impedance change $(Z_{cell} - Z_{No\ cell})/ Z_{No\ cell}$ vs. the log scaled frequency, measured by Solartron 1260 at 37 ºC. The peak of the input signal is 50 mV.

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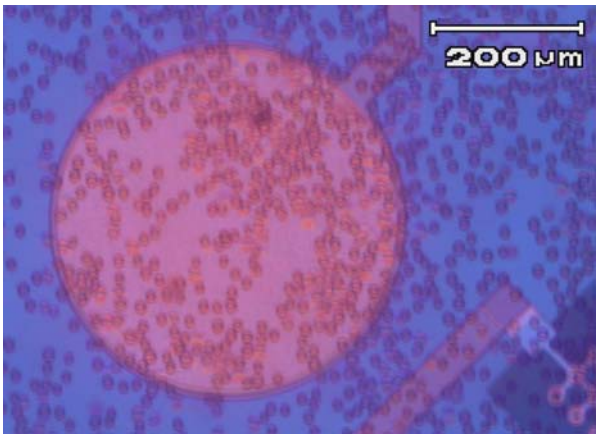
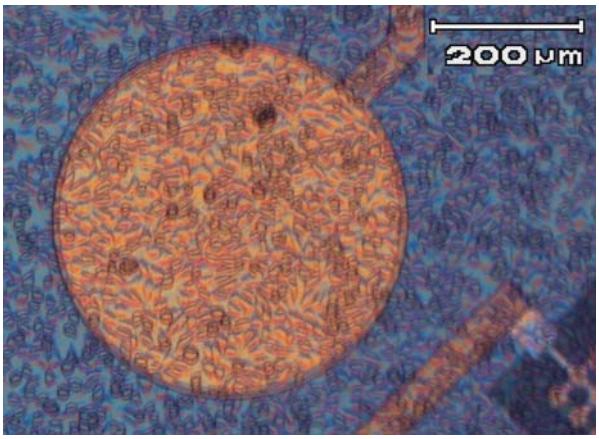
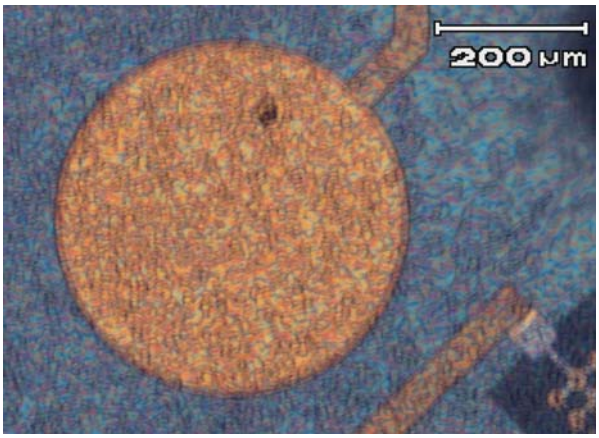


(a)



(b)

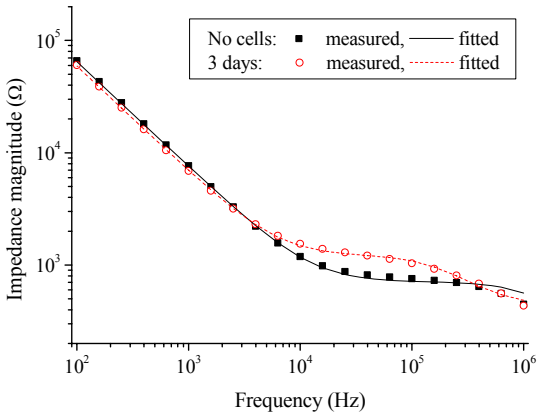


(c)

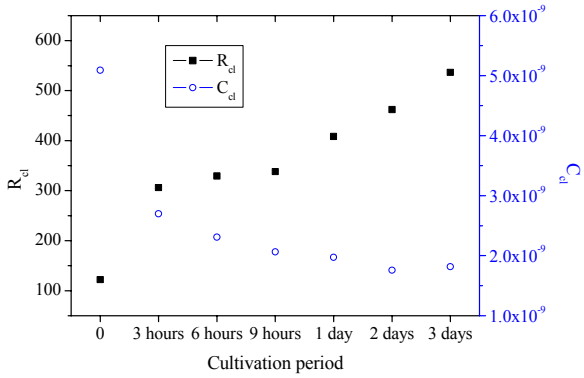
Figure 4: L929 cells on the sensing electrode (a) after pouring the cells or cultivation for (b) 3 hours or (c) 3 days.

Discussion

With electrode based cell chip and impedance measurement system, the cell growth can be monitored quantitatively. However, the effective frequency range for the *in vitro* monitoring with this system is limited by the electrode polarization at the low frequency and stray capacitance at the high frequency. Therefore, the



(a)



(b)

Figure 5: (a) Fitting the equivalent circuits in Figure 2 to measured impedance magnitude when no cells on the electrode (No cells) or after cultivation for 3 days (3 days), (b) R_{cl} and C_{cl} of Figure 2(b) according to the cultivation period, fitted by a software (Scribner Associates ZView).

electrode based cell chip and measurement system should have the proper electrode size and the low C_{stray} for the successive impedance monitoring of cell growth.

From the fitting results, it is found that the equivalent circuit can be used to analysis the impedance related to the cell growth on the electrode quantitatively. As the cells grow, the cells more adhere and spread onto the substrate [7]. R_{cl} and C_{cl} of the equivalent circuit can be parameters to indicate the cells adhesion, spread, and proliferation on the sensing electrode.

It is expected that the automated *in vitro* cell monitoring system using the electrode based cell chip and impedance spectroscopy will be more utilized in cell and tissue engineering.

Conclusions

Using the planar electrode structure and monitoring system, we investigated the impedance of L929 cell layer on the electrode during the cultivation. At various

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culture periods, the measured impedances were compared with the pictures about the cell layer. By fitting the equivalent circuits to the measured impedances, the growth of L929 cell layer on the electrode was monitored quantitatively.

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