Influence of transfection process on single cell impedance

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Abstract. Many new therapeutic approaches are based on gene transfection of cells. Data about dynamic interaction with cell membranes can support the selection of polymers and polyplexes regarding an optimal gene delivery reagent. A microchip/-fluidic system and an impedance-based method for monitoring the effects on individual cells after application of polymeric DNA-delivery systems is developed and evaluated. It was successfully demonstrated that the effects of polymers on the cell membrane can be monitored over time by using impedance measurements.

1. Introduction
Many new therapeutic approaches are based on gene transfection of cells. However, for an optimisation of gene transfection processes methods are needed urgently which can provide information about the interaction of gene delivery systems and cells. A fundamental property of a delivery system is that the gene vectors are able to cross the cell membrane and enter the intracellular space. It was found, that cell membrane damages can be monitored by impedance measurement ([1-3]). Thus it is assumed that the specific interaction with the cell membrane during a transfection process can be monitored by a suitable impedance-based setup.

Different impedance-based methods have been developed on the basis of Micro-Electro-Mechanical Systems (MEMS) in order to manipulate, handle or position single living cells and measure the electrical signal by various microelectrode interfaces. Some well known principles are microfluidic channels with integrated electrodes [4], micro electrode-based chips [5] and cell-filters [6]. These systems are engineered for instance for label-free cell discrimination or for basic studies of electrical and dielectrical properties of biological cells respectively. However, until now there is no existing setup suitable for a sensitive analysis of cell membrane alterations on single cell level in long-term measurements. To address this problem an impedance-based microfluidic unit predicated on a micro hole cell chip [7], [8] is developed. After hydrodynamical positioning of a single cell on a micro hole of a chip the cell adheres onto the biocompatible insulating chip membrane under cell culture conditions.

In this paper the setup for single cell impedance monitoring is evaluated regarding its suitability for measuring the interaction of gene transfer systems and cell membranes according to time. Therefore experiments are carried out with transfecting and non-transfecting polymer/DNA-complexes (polyplexes) as well as pure polymers. To validate the impedance measurements the data are correlated to standard molecular biological methods.
2. Methodology
For the measurement the micro hole cell chip described in detail in [7], [8] was used. The cell chip was integrated into a microfluidic biocompatible cell positioning unit. This micro-scaled unit consisted of one fluidic connection nanoport as well as two gold electrodes. The electrode interface was electrically linked to an impedance analyzer (Solartron 1260 and 1294, Solartron Analytical, Farnborough, UK) for the time monitoring. The two-terminal configuration turned out to be an adequate method for the measurement at 1kHz according to time, because the electrode polarization was negligible at this frequency. The applied input potential was set to 5mV. For single cell positioning on the micro hole a fluidic controller was used. This equipment consisted of a negative pressure system and a pressure sensor. To realize the cell culture conditions the impedance-based microchip system was put into a cultivation chamber combined with an integrated inverse microscope. The temperature, humidity and CO2 were adjusted to 37°C, 80% and 5% respectively. For the experiment, Arpe-19 (retinal pigment epithelial cell line) cells and a culture medium (DMEM/F12 31330, 10% FCS, 1% Penicillin/Streptavidin) were prepared. The schematic of the measurement setup is shown in figure 1.

3. Results and discussion
3.1. System stability
Figure 2 shows the positioning/adhesion process (figure 2(a)) and the system stability (figure 2(b)).

In figure 2(a) the low impedance magnitude level (< 125 kΩ) until 3.3 min described the unoccupied micro hole setup. At 3.3 min the cell was sucked on the hole, which increased the impedance magnitude level to a value around 180 kΩ. After the positioning the cell started to adhere to the biocompatible chip membrane surface, so that the impedance magnitude increased continuously till it reached a stable and maximum value (~ 350 kΩ).
During the experiments it pointed out, that an equilibrium state and therefore a constant impedance level of this cell line was given 14 min after the cell-positioning. Consequently a reasonable time to start monitoring was 14 min after the cell-positioning ($t_{14\text{ min}}$). Therefore an impedance normalisation was done as following.

$$
\frac{Z}{Z_{14\text{ min}}} = \frac{|Z| \exp(i \varphi)}{|Z_{14\text{ min}}| \exp(i \varphi_{14\text{ min}})} = \frac{|Z|}{|Z_{14\text{ min}}|} \exp\left[i \left(\varphi - \varphi_{14\text{ min}}\right)\right]
$$

Figure 2(b) shows that a stable impedance level could be guaranteed for more than 3 hours. The mean impedance magnitude values in figure 2(b) varied with 3.12% in the worst case situation. This variation could not be totally reduced, because of the cell motility on the hole and the morphological changes. The deviation in the stability was an important factor to find out which impedance alteration was caused by the substances or by the movement or the morphological changes of the captured cell.

3.2. Measuring membrane interaction on single cell level

The application of different polymers and polyplexes was done by exchanging the whole cell culture medium in the upper chamber by the polymer or polyplex solution. The time $t_{\text{application}}$ in the figures was the point of medium/(polymer or polyplex) exchanging.

Figure 3. Comparison of the normalized impedance magnitude between the control (from figure 2(b)) and the influence of the applied V01 (a) and VT01 (b) solution on the trapped cell.
Figure 4. Comparison of the normalized impedance magnitude between the control (from figure 2(b)) and the influence of the applied VT09+DNA (a) solution on the kept cell. In (b) three Propidium iodide (PI) positive Arpe-19 cells (one covers the micro hole) are shown 145 min after VT09+DNA application (stained nuclei are marked with white arrows).

The measured data in figure 3 and 4 showed at time $t = t_{\text{application}}$ that $|Z| \approx |Z|_{14\text{min}}$. Therefore the replacement of medium with the polymer or polyplex solution had no influence on the impedance level. In figure 3(a) no considerable difference in the impedance magnitude between the control and the polymer V01 could be observed. Consequently this reagent solution had no possibility to interact with the cell membrane. Figure 3(b) and 4(a) exhibited a significant decay in the impedance magnitude between the polymer VT01 and the control and the polyplex VT09+DNA and the control after 30 min and 60 min respectively. These data were correlated to the standard biological marker PI to validate the measurement and to figure out the reagent impact on the cell membrane. Figure 4(b) shows three PI positive cells, which means that the polyplex VT09+DNA was able to permeabilize the cell membrane. This membrane permeabilization caused the impedance level decay.

Based on the shown figures it was possible to conclude that the impedance-based micro hole chip setup is a highly stable and suitable method for measuring cell membrane alterations. It was successfully demonstrated that such an arrangement can be used for the selection of a polymer or polyplex concerning an optimal cell membrane crossing.

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