Impedance spectroscopy as a tool for real-time assessment of stem cell differentiation – a case study using neural stem cells

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Application of stem cells in different research areas, including biomedical engineering, has received an extensive focus during the past years. However, considering the diverse origin of stem cells, the protocols and biochemical processes involved, leading to the desired phenotype, vary from application to application. This presentation will focus on differentiation of neural stem cell models that have been designed to generate dopaminergic neurons applicable for cell replacement therapy of Parkinson's disease. To characterize the dopaminergic properties of differentiated neural stem cells, we have designed interdigitated electrode arrays that allow real-time monitoring of the cells' ability to release dopamine upon cell membrane depolarization. The designed electrode arrays have been used in both batch-based and microfluidic devices that facilitate initial cell culturing followed by cell differentiation. The goal to monitor the progress of stem cell differentiation on the electrode arrays without a need for continuous microscopic observations led to the interest in developing an impedance-based assay that would allow to assess the time point when the cell differentiation had been completed as well as to determine what electrodes in an electrode array had a fully developed cell population ready for monitoring dopamine release. Differentiation of neural stem cells starts from a population of adherent fusiform cells at a low cell density. Exposure to epigenetic factors leads to extensive formation of a dense neurite network that is not adherent to the culture substrate. The obtained results indicated that unlike our other applications of impedance-based monitoring of cellular behavior related to confluent monolayers of cancer cells (cytotoxicity of chemotherapeutic substances [1], effect of xenoestrogens [2], and cell invasion [3]), differentiation of neural stem cells required acquisition of complete impedance spectra combined with equivalent circuit analysis. Figure 1 shows the characteristic equivalent circuit model that describes the behavior of differentiating neural stems cells [4]. Our previous studies on the differentiation process of neural stem cells have shown the diverse aspects related to changes in gene expression, leading to synthesis of proteins that are characteristic of the differentiation outcome, i.e., dopaminergic neurons. However, from the point of view of impedance-based monitoring of cell differentiation, the obtained results indicated that the primary aspect that led to the characteristic impedance behavior was related to the distinct morphological changes when the neural cytoskeleton, composed of β-tubulin, was being formed. Overall, we can conclude that impedance-based monitoring can reveal details of stem cell differentiation and serve as a tool to assess the progress of differentiation. However, each type of stem cell application and type of stem cell requires thorough characterization of impedance behavior during differentiation. No generic impedance assay protocol automatically fits all stem cell applications.

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