Testing Procedures for Safe Cell Stimulation Parameters with Micro-Electrodes Using Living Cells

Krueger TB 1, Becker S 2, Hoffmann KP 2, Stieglitz T 1

1 Laboratory for Biomedical Microtechnology, IMTEK - Institute for Microsystem Technology, University of Freiburg, Georges-Koehler-Allee 102, 79110 Freiburg, Germany
2 Department of Medical Engineering and Neural Prostheses, IBMT - Fraunhofer Institute for Biomedical Engineering, Ensheimer Str. 48, 66386 St. Ingbert, Germany

E-Mail: krueger@imtek.de

Abstract

Fabricating micro-electrodes with new procedures it is not clear in advance how the characteristics of the designed electrodes will be. To avoid time consuming and possibly unethical procedures in-vivo, we are introducing a combined test method of in-vitro studies and cell-on-a-chip experiments. Electrodes were first characterized in-vitro by impedance spectroscopy, and then tested for long-term stability with stimulation pulses. Cells were cultivated on the electrode design. With confluent cell layers, implying the biocompatibility of the chosen materials, chronic stimulation of the electrodes has been undergone. The electrochemical limit of the maximum charge transfer capacitance of the electrodes was determined. As well as the tolerable stimulus injection limit for avoiding cell damage or even cell death. The results give a clear outlook of the electrode behaviour in the living environment, its later destiny.

1. INTRODUCTION

For the biomedical application field of batch fabricated micro-electrodes in biological environments it is mandatory to know the long-term characteristics and environment influencing factors of the device. Electrical characteristics like charge capacity and impedance can be easily obtained in the laboratory with quasi standardized experiments, whereas both, pre-design simulation studies and biocompatibility tests are rarely used. We will introduce two methods for checking micro electrodes towards their later purpose for enhancing knowledge of designated electrodes.

2. METHODS

Due to the different applications phases two different method areas of characterization can be clearly distinguished: preliminary theoretic considerations including electrical measurement and living cell environment.

2.1. Electrical Methods

First the theoretic charge delivery capacitance of each used electrode was calculated, assuming a maximum transfer capacitance for platinum of 75 µC/cm² ranging below known in-vivo values [1](Figure1). Electrode impedance spectroscopy was done with an impedance analyzer with electro-chemical interface (Solartron 1260 and 1287, Farnborough Hampshire, UK) in a three electrode setup (PT1800 counter electrode, Ag/Ag-Cl reference electrode, Schott, Mainz, Germany) in Ringer-Solution. The electrodes were characterized as working electrode.

![Figure 1: Theoretic calculated maximum charge deliverance capacitance with 100 µs pulse width.](image)

To apply electrical stimulation to the electrodes either a handheld battery powered stimulator was used for very low voltage or current rectangular pulses, or a computer driven pulse generator to apply greater potentials over a long time period. Stimulators were self-developed at
the IBMT and customized for the application area of micro-electrodes.

Stimulating parameters were induced in the range of 0.05 mA up to 0.5 mA, pulse widths were chosen between 20 µs and 200 µs. Induced charge transfer ranged from 0.001 to 0.1 µC per phase. Electrode impedance was measured at all time points of interest. Used electrodes were sited on a multi electrode array (MEA) developed at the IBMT, called 2D-Heart-Cell-Chip, providing 40 electrodes with both 30 µm and 50 µm in diameter for measurement purposes and 10 counter electrodes with the diameter of 500 µm. Electrodes consisted of platinum enhanced with platinum-black for lower impedance. Further materials used in the production of the MEA with direct cell contact were Si₃Ni₄ for the insulation layer and medical grade Silicon.

2.2. Cell Environment

According to the international standard ISO 10993, which recommends L929 fibroblast cell lines for biocompatibility test, this cell line was used for initial testing of the set-up. More sensitive, primary cells from the dissociated heart of the six day old chicken embryo were put directly on the MEA. Using DMEM/F-12 medium at 37 °C, 95 % relative humidity and 7.5 % CO₂ confluent cultivation of the heart cells was made. The behaviour of the cells was permanently optically supervised. Easily the state of the cells could be stated by spontaneous periodic contraction. Stimulation was best applied on centers were the intensity of the cell activity was at its peak. Two different incubators were used to avoid cross-contamination of the sterile area of cell cultivation from the less sterile area of stimulation including the electrical devices. Meanwhile the stimulation a temperature controlled chamber was used adjusted to 37 °C but without CO₂ and humidity control.

3. RESULTS

In the laboratory 10 MEAs were characterized, with each having 40 small diameter electrodes and 10 counter electrodes. Four systems underwent in-vitro stimulation testing. Mean impedances were at 10 kΩ at 1 kHz and did not change while safe stimulation (Figure 4). Total charge transfer from 24 µQ up to 180 µQ did not changed the electrode behaviour neither induced damage. Gaining the safe stimulation limits for the electrodes cell experiments with the cell line L929 were started, leading to confluent cell layer showing the good biocompatibility of the MEA. Stimulation tests with this cell line resulted in no dead cells, but no graded sensitivity during subletal stimulation was possible, caused on a missing suitable test method. Therefore 7 MEA were successfully cultivated confluent with living heart cells, not only the biocompatibility of the materials was proved with this second cell line, also graded stimulation series were started. All MEAs had sufficient confluent cell layers (Figure 2).

In different regions of the cells centers of palpitation could be observed. Different sets of stimulation were executed, a total of 14 different stimulation parameters were applied and in addition several experiments were repeated for verifying the obtained results (Table 1). Summarizing the data into one main parameter, the total charge transfer, the clear line of beginning cell death could be observed beginning with 270 µC with 50 µm diameter electrodes (Figure 3).

4. DISCUSSION

With the ongoing experiments the range of safe stimulation could be determined for micro-electrodes. Previous results made in different publications with a variety of electrode areas could be replicated - even emerging in a nearly proportional charge transfer capacitance [1][2][3][4].
The theoretic knowledge could be transferred towards the scale of the micro-electrode; although the critical parameters where irreversible cell or electrode damage comes, are clearly earlier than the scaled values derived from macro-electrodes are suggesting. With a sufficient stimulation time lasting at least half an hour the cells could be brought in unison with the applied stimulation frequency of 1 Hz. So the influence of stimulation was clear and pacemaker theory could be repeated in-vitro with an artificial sinu-atrial node.

The threat for the biological environment is not always directly coming from the electrical stimulation itself but sometimes indirect of possible effects, especially electrode corrosion. When electrodes are driven beyond their designated use area, ‘Blown’ electrodes can contaminate the near environment and induce so a strong reaction or even cell death.

A homogenous way to characterize and supervise micro-electrodes could be shown, starting at the first idea of the electrode until it is produced and ready to use.

Figure 4: Impedance of 23 electrodes with 30 µm in diameter, the influence of 50 Hz cycle noise is obvious.

References

Acknowledgements
We want to thank the Fraunhofer Institute for Biomedical Engineering for their generous possibilities giving to us for these experiments. Special thanks are going to the department Biohybrid Systems facilitating the cell studies.