Pulse-clamp technique for characterisation of single-cell stimulation electrodes

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A reliable test method is required to evaluate charge injection capability for neural stimulation applications that demand both a large amount of charge injection and a small electrode size. The pulse-clamp method is well suited for analysis of electrode materials, since the time scale is equal to that used for the stimulation procedure. Simulations were performed and compared to experimental results from measurements of sputtered iridium oxide film (SIROF) electrodes. Results show that the model correlates well with experimental data. This approach can be used for the characterization of minaturised stimulation electodes.

The design of novel bidirectional interfaces for *in vitro* single neuron networks is an important step towards the use and understanding of neuronal coupling to electronics and neuronal enhanced signal processing in biohybrid circuits. Such devices typically feature electrodes based on either platinum, titanium nitride, or iridium oxide (IrOx). A number of reports documenting the use of IrOx microelectrodes for neuroengineering and biomedical applications have been presented [1-3]. The interest in this material is driven by its excellent properties as a functional coating for implantable stimulation electrodes, with applications in stimulating/recording of heart, neuronal or retinal tissues. Due to its fractal surface morphology, the IrOx's capacity for charge storage is significantly higher compared to platinum. This also results from a fast and reversible faradaic reaction which involves reduction and oxidation of the oxide [4].

Since the traditional electrochemical tests, i.e. cyclic voltammetry (CV) and impedance analysis, do not operate at the same time scale or voltage amplitudes as is required in neural stimulation, they are inadequate to investigate the true electrode dynamics. Therefore, a reliable test method is needed to evaluate the charge injection capability at the neuron/electrode interface, since high resolution neural stimulation demands both a large amount of charge injection and a small electrode size. These requirements are met by the pulse-clamp method which was introduced by Mortimer and co-workers [5] and also used by Hung *et al.* [6].

The focus of this paper is to examine and discuss the use of the pulse-clamp technique to characterize single neuron stimulation electrodes. We adapted and improved a pulse-clamp circuit which operates similar to the setup used earlier by Mortimer et al. [5] that achieved stable high-speed operation on low current values to characterize and compare electrodes well below 300 µm in diameter for the first time [7-9].

Planar microelectrode arrays (MEAs) were fabricated by sputtering of an iridiumoxide layer on gold elecrodes as described in [7] by using a Nordiko NS2550 magnetron sputtering tool (Control Process Apparatus Inc., Fremont, CA, USA) with the following parameters: 180 W DC power, 100 sccm argon flow and 10.4 sccm oxygen flow. A *3* µm thick layer of parylene-C (deposited with a Specialty Coating Systems PDS 2010 Lab-coter) was used as an insulating layer, covering the leads while leaving free access to the electrodes sites and contacts. Electrodes with different diameters are shown in Fig. 1.

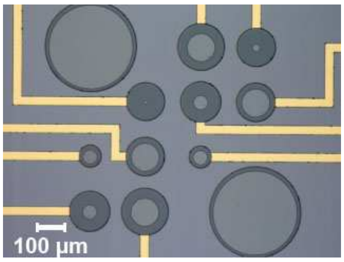

FIG. 1: *Planar IrOx microelectrodes of different sizes*

The principle of the pulse-damp method is to perform a discharge current measurement with a high time resolution after an electrode has been charged by a current pulse, as shown in Fig. 2.

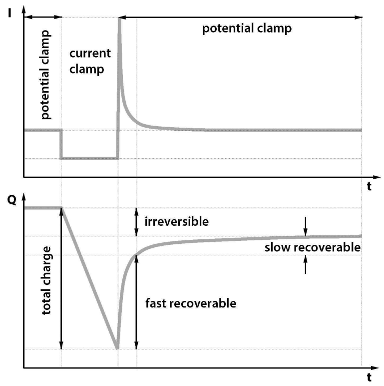

FIG. 2: *Current and charge behaviour during pulse-clamp experiment*

First the working electrode is set to a potential, e.g. 0 V vs. the reference electrode. During testing, the instrument switches to current mode and forces a constant current (cathodic or anodic), simulating half of a neural stimulation pulse sequence, called the current clamp (CC). Directly after this current pulse the potential of the stimulation electrcode is fixed to the same value as before the pulse, instead of immedialtely following with a second current pulse of equal charge and opposite polarity. This stage is called the potential clamp (PC). As shown in Fig. 2, a certain amout of charge is injected during the CC, followed by a discharging phase during the PC. Three different stages in the discharge phase can be identified [5]: the fast discharge directly after the CC, correlated to fast recoverable electrochemical processes, the slow discharge, based on slow recoverable processes, and a certain difference of charge which cannot be recovered due to being stored in permanent products of faradaic processes, the unrecoverable charge.

All experiments were carried out on planar SIROF microelectrodes. Phosphate buffered saline (PBS) solution with 154 mM NaCl was used as the electrolyte in all experiments. The setup used consists of the improved pulseclamp circuit and a National Instruments NI USB-6125 device for data acquisition at a sample rate of 1.25MS/s and a resolution of 16bits, which complies with demands set by the pulse-clamp circuit regarding speed and resolution. Using a custom designed LabVIEW program the current was measured and the corresponding charge was obtained by calculating the time integral. The current pulse width was kept constant at 400 µs and the magnitude of the current during the CC was adjusted to ensure that no water electrolysis or electrode dissolution occurred. All experiments where performed within the charge injection limits of 1 - 5 mC/cm^2 in the safe potential window of -0.6 V -0.8 V vs. Ag/AgCl.

Figure 3 illustrates the measured charge of two SIROF microelectrodes, one having a 100 µm diameter (approx. area of 7850 µm^2) and the other 25 µm (approx. area of 500 µm^2), during a pulse-clamp experiment.

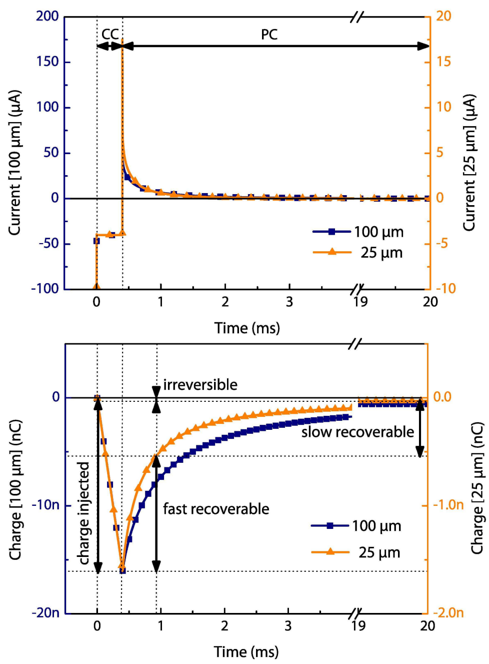

FIG. 3: *Measured current of SIROF electrodes with different sizes and its correlated charge retrieved by integrating over time, after [9]*

A cathodic current pulse of 400 µs was applied to the electrodes maintaining an equal charge density per area of approx. 0.12 mC/cm^2. This results in a total injected charge of 10.13 nC for the 100 µm and 507 pC for the 25 µm electrode. The amount of charge recovered in this process is higher than 94% for both electrodes. Comparing the discharge stages of the two electrodes, it can be observed that the 100 µm electrode has a slower discharge compared to the 25 µm one. Most likely this is due to different capacitances and therefore different RC time constants.

The scalability of the pulse-clamp circuit allows it to be used to accurately quantify the quality of a surface modication for microelectrodes well below 300 µm in diameter. By applying the pulse-clamp method it can be seen that when very low charge densities are applied nearly no charge loss is present due to the double-layer capacitance charge storage or reversible redox reactions. However, the charge loss cannot be totally avoided as has been shown before by Brummer and co-workers [13]. The pulse-clamp results of SIROF electrodes of different sizes show charge losses less than 6%, and a superior reversible charge injection capability of SIROF compared to platinum microelectrodes of the same size, even at higher charge density levels. The pulse-clamp method allows an accurate electrode parameter extraction and a comparison of the charge-injection capabilities of different electrode sizes and materials.

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