

# In Vivo Charge Injection Limits Increased after ‘Unsafe’ Stimulation

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**Abstract:** The effect of unsafe stimulation on charge injection limits ( $Q_{inj}$ ) and pulsing capacitance ( $C_{pulse}$ ) was investigated. Four stimulation protocols were applied: 20 mA – 200 and 400 Hz, 50 mA – 200 and 400 Hz. Increasing  $Q_{inj}$  and  $C_{pulse}$  were observed for all stimulation protocols. Corrosion was not observed with any of the stimulation protocols and no tissue damage was observed for the 20 mA – 200 Hz stimulation group. This indicates that the ‘safe potential window’ may not be applicable *in vivo*, as no damage was done stimulating with 20 mA at 200 Hz, while damage was done using the same current at 400 Hz.

## 1 INTRODUCTION

The performance of stimulation electrodes can be characterized by their charge injection limits ( $Q_{inj}$ ). The amount of charge that can be injected safely without causing electrode degradation or tissue damage depends on the electrode material, the electrolyte and the stimulation waveform. Typically, the potential limits for safe stimulation are determined under near steady-state conditions using the cyclic voltammogram (CV). The CV reveals how the electrode material interacts with the electrolyte or the tissue at stepwise in- and decreasing potentials. The safe potential window typically is defined by the potentials at which water reduction and oxidation occurs. (Cogan, 2008)

The safe potential window and  $Q_{inj}$  are typically reported under *in vitro* conditions, in inorganic saline solution. Both the safe potential window (Meijs, submitted), as well as  $Q_{inj}$  (Kane, 2013; Wei and Grill, 2009; Meijs, submitted) differ under *in vivo* circumstances. Furthermore,  $Q_{inj}$  and electrode polarization change during the course of the implanted period (Kane, 2013; Lempka, 2009; Meijs, submitted).

In order to investigate the reliability of the *in vivo* charge injection limits, electrical stimulation was performed for 6 hours in anesthetized animals using charges that exceeded  $Q_{inj}$ . Six hours of stimulation at 200 Hz using a 20 mA current caused no tissue or electrode damage during a pilot study and this was therefore used as the least intense

stimulation paradigm. Three other stimulation paradigms were added by doubling the frequency and increasing the current to the maximum stimulator output (50 mA). During stimulation voltage transients (VT) were recorded and charge injection limits were measured before, during and after the stimulation period.

## 2 METHODS

Four pigs were implanted with 4 porous TiN working and 4 pseudo-reference electrodes of the same material each. The work was carried out according to Danish legislation (ethical approval license nr: 2014-15-0201-00268).

### 2.1 Electrode Fabrication

TiN coatings were deposited on Ti6Al4V electrode pins (6 mm<sup>2</sup>) and Ti disks (1000 mm<sup>2</sup>) by reactive magnetron sputtering on a CC800/9 SiNO<sub>x</sub> coating unit (CemeCon AG, Germany). The electrodes were mounted on a rotating stage, which carried out a three-fold planetary rotation. Sputtering was done from four Ti targets (88 x 500 mm<sup>2</sup>) with 99.5% purity in a Ar/N<sub>2</sub> mixture atmosphere. The purity of both gases was 99.999%. An ETFE coated 35N LT wire (Heraeus, Switzerland) was crimped to the hollow end of the electrode pins. The pins were insulated using a PEEK body with silicone tines, which were glued to the pins. The electrodes were

cleaned thoroughly before e-beam sterilization.

## 2.2 Surgical Procedure

The animals were first sedated and then anesthetized with a bolus injection of propofol. Anesthesia was maintained using propofol infusion. The electrodes were implanted in tight pockets in the subcutaneous adipose tissue on the back. The percutaneous electrode wires were encased in surgical tape, which was sutured to the skin. The electrodes were not used for one month until the pigs were anesthetized again using sevofluran to perform electrical stimulation.

## 2.3 Electrical Stimulation

Biphasic, charge balanced 200  $\mu$ s square pulses were applied, cathodic first with an inter-phase interval of 40  $\mu$ s during which no current was applied. Stimulation was performed for 6 hours, which was divided into 3 2-hour sessions. Before, between and after these sessions,  $Q_{inj}$  were determined for each electrode. Four stimulation paradigms were applied:

- 20 mA, 200 Hz
- 20 mA, 400 Hz
- 50 mA, 200 Hz
- 50 mA, 400 Hz

Electrical stimulation was performed using DS5 (Digitimer, UK), which was shorted between the pulses. VT were recorded using an oscilloscope.  $Q_{inj}$  were measured using the VersaSTAT 3 potentiogalvanostat (Princeton Applied Research, USA).

The pulsing capacitance ( $C_{pulse}$ ) was computed using the slope ( $\frac{dV}{dt}$ ) of the VT:

$$I_{stim} = C_{pulse} \frac{dV}{dt} \quad (1)$$

Where  $I_{stim}$  is the stimulation current.  $Q_{inj}$  was calculated using the current at which the safe potential limits (-0.6 and 0.9 V) were reached ( $I_{max}$ ):

$$Q_{inj} = \frac{I_{max} \cdot t}{A} \quad (2)$$

Where  $t$  is the duration of the stimulation pulse (200  $\mu$ s) and  $A$  is the geometrical surface area of the electrodes (6 mm<sup>2</sup>). When voltage excursions exceeded machine limits ( $\pm 10$  V),  $I_{max}$  was extrapolated from the highest current using a linear relation.

$$V_{ext} = V_m \left( 1 + \frac{I_{ex} - I_m}{I_m} \right) \quad (3)$$

Where  $V_m$  and  $I_m$  were the measured potential and current, respectively, and  $V_{ex}$  and  $I_{ex}$  were the ex-

trapolated potential and current. When  $V_{ex}$  reached the potential limits,  $I_{ex}$  was used as  $I_{max}$  in (2). This method provided accurate results using data for which  $I_{max}$  was measured.

## 2.4 SEM/EDX

SEM (Nova 600, FEI Company) images were recorded at various magnifications to investigate the surface structure of the electrodes. EDX (EDAX, AMETEK) spectra were made to determine if there the surface chemistry of the electrodes changed.

## 3 RESULTS

During the measurements the shorting part of the setup broke down, and the last two stimulation sessions could not be done with one of the electrodes in the 20 mA – 400 Hz group.

The average  $Q_{inj}$  of all implanted electrodes before stimulation was  $12.3 \pm 1.4 \mu\text{C}/\text{cm}^2$ . After 2hrs of stimulation  $Q_{inj}$  was increased for all of the individual electrodes (Fig. 1) and the largest increase was observed for the group 4.

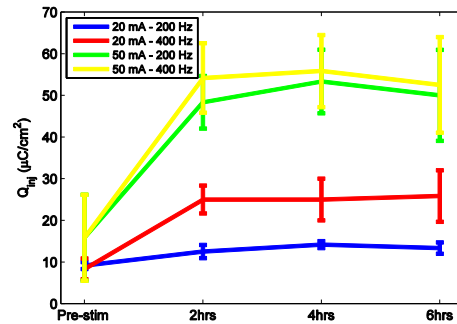


Figure 1:  $Q_{inj}$  was increased after the first stimulation session. It then remained stable for each electrode group.

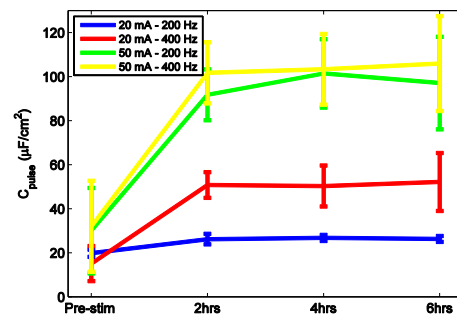


Figure 2:  $C_{pulse}$  was increased after the first stimulation session. It then remained stable for each electrode group.

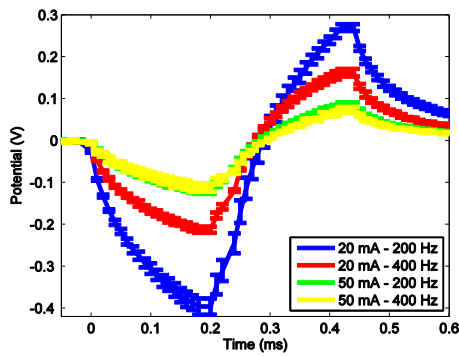


Figure 3: After 2 hrs of stimulation, the slopes of electrode groups were decreased with increased charge injection, due to the increased  $C_{pulse}$ .

The average  $C_{pulse}$  of all implanted electrodes before stimulation was  $24 \pm 2 \mu\text{F}/\text{cm}^2$ . Fig. 2 shows an increase of the average  $C_{pulse}$  of all electrodes by approximately the same relative amount as  $Q_{inj}$ . Fig. 3 shows that the slopes of the VTs were decreased due to the higher  $C_{pulse}$  after 2 hours of stimulation.

Analysis of the VT during the stimulation sessions showed that  $C_{pulse}$  increased after the first 30-60 minutes of the first stimulation session, but it did not increase during the second or third session (fig. 4). Furthermore,  $C_{pulse}$  was increased for all stimulation groups as compared to  $C_{pulse}$  derived using a safe stimulation current (fig. 2).  $C_{pulse}$  was also significantly higher at 50 mA than at 20 mA.

SEM showed that the electrode surfaces were intact after 6 hours of intense stimulation. Similar levels of oxide were observed on all electrodes using EDX.

## 4 DISCUSSION

Although  $Q_{inj}$  increased from the initial level depending on the stimulation current and frequency, the charge injection was always higher than  $Q_{inj}$ .

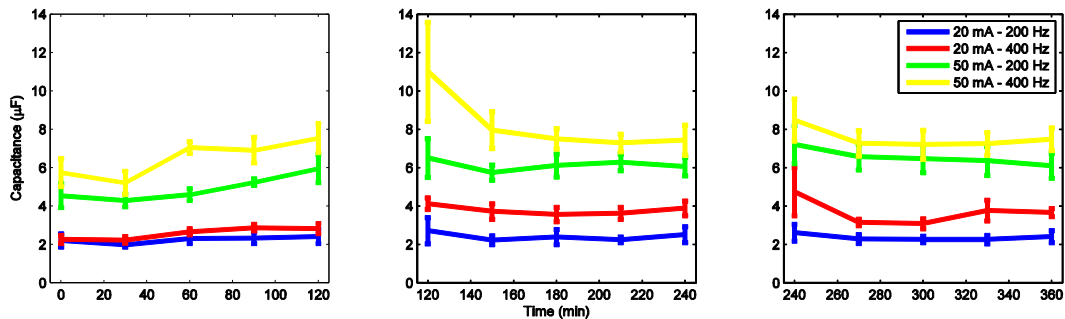


Figure 4:  $C_{pulse}$  increased during the first stimulation session for electrodes for which tissue damage was observed.

Above this theoretical limit, tissue and/or electrode damage is expected (Cogan, 2008). Neither of these was observed for the electrodes stimulated at 20 mA – 200 Hz. Increasing levels of tissue damage were, however, observed with increasing charge injection in accordance with findings of Mortimer (1980). Tissue damage was due to the accumulation of a reaction product to detrimental concentrations or pH changes beyond the buffering capacity of the tissue, as heat damage, the mass action theory and corrosion were ruled out (Shannon, 1992; Merrill, 2005).

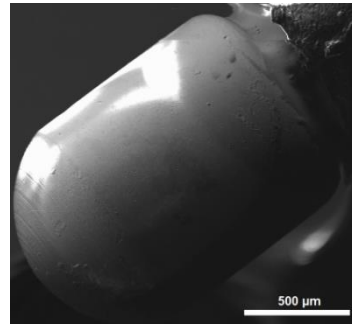


Figure 5: SEM image of an electrode from group 3.

Most plausible seem changes in the pH, as water reduction occurs in saline at -0.6 V for TiN, resulting in increased levels of  $\text{OH}^-$ . Electrode potentials were below -1 V for all electrodes at the start of the first stimulation session. Water reduction is likely to have contributed to charge transfer during all stimulation protocols, as  $C_{pulse}$  derived from the voltage transients during the stimulation sessions (0 min in fig. 4) was doubled for 20 mA electrodes and more than tripled for the 50 mA – 400 Hz electrodes as compared to  $C_{pulse}$  derived at 1 mA (pre-stim in fig. 2). This is likely due to the transfer of charge via water reduction, which is a faradic process and does not increase the electrode potential (Merrill, 2005). No complete levelling off of the potential was observed, however.

Furthermore, the slope of the voltage transients decreased towards the end of the cathodic phase, indicating that at increasing cathodic potentials, water reduction contributes more to the total charge transfer (Merrill, 2005). This trend was observed primarily during the first 30-60 minutes for the electrode groups for which damage was observed, but not for the 20 mA – 200 Hz group. After 30-60 minutes, the decrease in slope was less and it remained stable throughout the rest of the study. This makes it plausible that tissue damage due to stimulation induced pH changes occurred during the first 30-60 minutes of stimulation, which fits well with the pH changes observed in saline as a function of time (Mortimer, 1980).

The increase in capacitance that is observed after 30-60 minutes (fig. 4) for the electrode groups with tissue damage is likely due to destruction of the fibrous capsule. This removes the diffusion limitation, which typically limits the charge injection capacity of implanted electrodes (Cogan, 2008).  $Q_{inj}$  and  $C_{pulse}$  derived at safe stimulation levels (fig. 1 and 2) were increased more for electrode with than without tissue damage.

Electrode damage was not observed, though the average anodic potentials were above 1 V for all electrode groups and potentials of more than 2 V have been observed in all groups, except 20 mA – 400 Hz. Oxidation of the TiN surface into a thin oxide/oxy-nitride film occurs at 0.5-0.9 V. These processes lead to passivation and protect the underlying TiN from further oxidation. At higher anodic potentials (1-1.5 V) oxidation of the TiN into hydroxide and/or  $TiO_2$  occurs. Lastly, at potentials higher than 2 V, which have been observed in this study, oxygen evolution takes place accompanied by oxidation of TiN to  $TiO_2$ . (Avasarala, 2010) There are three reasons why we may not have detected increasing levels of oxide with increasing charge injection. 1) The oxidation reaction is reversed during the cathodic phase (Merrill, 2005). 2) The oxide has dissolved in the acidic environment (Avasarala, 2010) that was created due to intense electrical stimulation (Merrill, 2005). 3) The oxide levels on all electrodes are below the detection limit for EDX.

The increase in  $Q_{inj}$  and  $C_{pulse}$  when no tissue damage occurred is in accordance with a decrease in polarization resistance observed for cochlear implants (Tykocinski, 2005; Newbold, 2014) and a decrease in complex impedance of deep brain stimulation electrodes before and after stimulation (Lempka, 2009). Newbold (2014) argues that the stimulation induced changes are confined to the electrode tissue interface and that protein ad-

desorption may be responsible for them, as they saw no changes in the voltage drop that is due to resistive properties of the tissue (IR drop). For the 20 mA – 200 Hz group, there were no changes in IR drop and no tissue damage. For all other electrode groups, however, tissue damage was observed, as well as a decrease in IR drop.

All electrodes were capable of the same charge injection before stimulation was started yet tissue damage occurred in the 20 mA – 400 Hz group, but not in the 20 mA – 200 Hz group. This shows that a safe stimulation protocol for implanted electrodes is not only established by keeping within a certain potential window (Merrill, 2005) and that the ‘safe’ window is not necessarily applicable *in vivo*. Safe stimulation also depends on the stimulation frequency, as this limits the time of the tissue to restore the pH (Ballestrasse, 1985). There may be a safe amount of charge that can be injected regardless of the frequency. It is, however, difficult to determine this amount, as a purely linear voltage change was not observed even within the theoretical ‘safe window’. It would therefore be interesting to investigate whether tissue damage occurs using ‘safe’ stimulation currents at very high frequencies.

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