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In Vitro and *In Vivo* Charge Capacity of AIROF Microelectrodes

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***Abstract*—Activated Iridium Oxide Film (AIROF) microelectrodes are thought to be well-suited for neural stimulation of the cortex because they can sustain high charge capacity (about ten times higher than Pt microelectrodes) when characterized in phosphate-buffered saline (PBS) or other high ionic strength electrolytes. However, it is known that their capacity diminishes after they are implanted *in vivo*. It has been suggested that tissue encapsulation is an underlying cause. In this paper, we report electrochemical measurements of AIROF microelectrodes that were performed acutely in the brain of the zebra finch. The experiment showed that the interstitial fluid environment in the bird's brain did not maintain the high charge delivery capacity of the AIROF microelectrodes. A simple compensation for access resistance may create hazards to sustained electrode integrity.**

I. Introduction

Cortical visual prosthesis research, as well as other CNS electrical stimulation applications, often employ neural stimulating microelectrodes with surface areas on the order of 10^{-6} to 10^{-5} cm^2 to achieve high selectivity of neural stimulation [1]. Delivering the stimulation current with such a small area requires microelectrodes that are capable of very high charge density. Activated iridium oxide film (AIROF) microelectrodes are known to have advantages over other noble metal electrodes due to the reversible redox reaction (valance transition between Ir (III) and Ir (IV)) of the hydrous iridium oxide film that facilitates charge delivery. It is not unusual to achieve an *in vitro* charge density of about 3-4 mC/cm^2, about ten times larger than what Pt microelectrodes can typically produce [2].

Though iridium oxide film electrodes have been intensively studied and characterized in well-controlled acid or basic electrolyte environments [3], few experiments have investigated these electrodes in the interstitial fluid environment, which contains ions and complex organic compounds that are very different from, and difficult to imitate, in the *in vitro* environments. Several chronic neural recording and stimulation experiments using AIROF microelectrode arrays have evaluated the stability of this electrode-neural interface [4], [5]. One observation is that the charge capacity of the electrode diminishes greatly after being implanted *in vivo*. Though the mechanism is not yet clear, for seemingly similar effects of recording electrodes, conditioning methods were suggested to "revive" the electrode/tissue interface [6].

In this paper, we report cyclic voltammetry (CV) and compliance limited [7] current pulse measurements that were made on the same AIROF microelectrodes that were repeatedly implanted into and explanted out of the brain of an anesthetized zebra finch over a short period of time. The experiment showed that the AIROF microelectrodes could not deliver as much current in the interstitial fluid environment of the bird's brain as in the *in vitro* electrolyte.

II. Materials and Methods

A. Microelectrodes

Pure iridium microelectrodes were purchased from Micro Probe Inc. (Potomac, MD) in single unit form. The Parylene-C insulation along the shaft was ablated by laser to expose the electrode tip (~2000 μm^2). Prior to activation, residual laser-ablation debris was removed from the metal surface according to the following cleaning procedure: 2-minute ultra-sonic in acetone, 2-minute oxygen plasma, 1-minute 7% HF dip, and another 2-minute oxygen plasma. The cleaning result was confirmed by examining the electrode under a 4200x digital microscope.

Then the electrodes were activated at 0.1 Hz (during which the iridium oxide film was grown) using a current driven method that current-pulses the electrode between 10 μA anodic current and 60 μA cathodic current while keeping the compliance voltage limit between +0.9 to –0.6 V vs. Ag|AgCl. Fig. 1 shows the images (taken by Hirox digital microscope system 4200x) of the two activated AIROF microelectrodes: *MPA06* and *MPA08*.

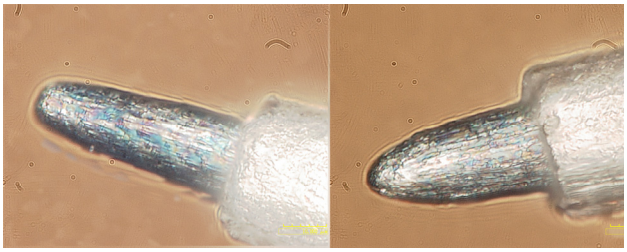

Fig. 1. Activated AIROF microelectrode (*MPA06* left; *MPA08* right). Both electrodes were activated and the chromic effect is caused by the thin layer of iridium oxide film over the bare iridium metal electrode. The shaft of the electrode is insulated with Parylene-C.

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B. Surgery

All animal procedures were approved by an Institutional Animal Care and Use committee at the University of Chicago. Adult female zebra finches (*Taeniopygia guttata*) were anesthetized with a 0.05ml injection of modified Equithesin and placed in a stereotaxic head holder. The skull was exposed, and a small hole was made over the rostral portion of the right hemisphere. An uncoated large-area Pt-Ir electrode was lowered into the brain to a depth of ~1000 μm and cemented to the skull with dental acrylic to act as the counter electrode. The upper shelf of the skull was removed over the caudal portion of the right hemisphere, and using a PBS solution bridge, the reference electrode was placed in contact with the exposed lower shelf. A small hole was then made over the caudal portion of the left hemisphere. The brain was left exposed at this location in order to implant and explant the AIROF electrodes before and after the in-vivo measurements were taken. AIROF electrodes were lowered into the brain to a depth of ~500 μm. The exposed brain surface was hydrated with saline as needed. At the conclusion of the experiment, birds were euthanized with a 0.10ml injection of Nembutal (50mg/ml).

C. Electrochemical Measurements

A three-electrode potentiostatic configuration was used for both *in vitro* and *in vivo* electrochemical measurements: working electrode, Ag|AgCl electrode as the reference electrode, and a large Pt mesh as the counter electrode *in vitro*; and, a Ag|AgCl electrode touching the skull with a salt bridge tube as the reference electrode, combined with a large-surface area uncoated Pt/Ir electrode implanted in the brain as the counter electrode. A custom-designed LabView program controlled the experiment procedure that switches automatically between the Gamry Electrochemical measurement system and our stimulus-current pulsing system. Each electrode was measured as follows:

1. CV at three different scan rates (50 mV/sec, 5 V/sec, 50 V/sec) and compliance-supply-limited current pulse measurements were taken on the AIROF microelectrode in phosphate-buffered saline (PBS) and 1/16 concentration PBS (NaCl 0.126M, NaH_2PO_4 0.0014M, Na_2HPO_4 0.005M) before implantation.
2. After the electrodes were implanted in the bird's brain, the same set of measurements was taken *in vivo*.
3. The electrode was explanted, washed briefly in water ultra-sonically, and the same set of measurements was taken again.
4. Go to step 1 and repeat this procedure for the same electrode.

III. Results

A. Current Pulse Measurements

Fig. 2 shows the current pulse waveform of AIROF microelectrode *MPA08* both in the bird's brain (middle column) and before (left column) and after (right column) the implantation. As shown in the figure, we biased the electrode at +0.6 V vs. the Ag|AgCl reference electrode. Then the cathodic current pulse drove the electrode voltage negative. Since the compliance limit of our system is kept within the water window (in this case ±0.6 vs. Ag/AgCl), the current is automatically cut back, to sustain the voltage for the pulse width of 300 μs.

It is obvious that in 1/16 concentration PBS, which we had thought would simulate the low ionic strength of the interstitial fluid, *MPA08* can deliver a peak current over 80 μA within the chosen compliance limit. However the same electrode (in both trials) cannot deliver over 20 μA current, for the same compliance limit, while in the bird's brain.

The same phenomenon happened on electrode *MPA06* as shown in Fig. 3. (The diminished current in the lower right plot of Fig. 3. will be explained below.)

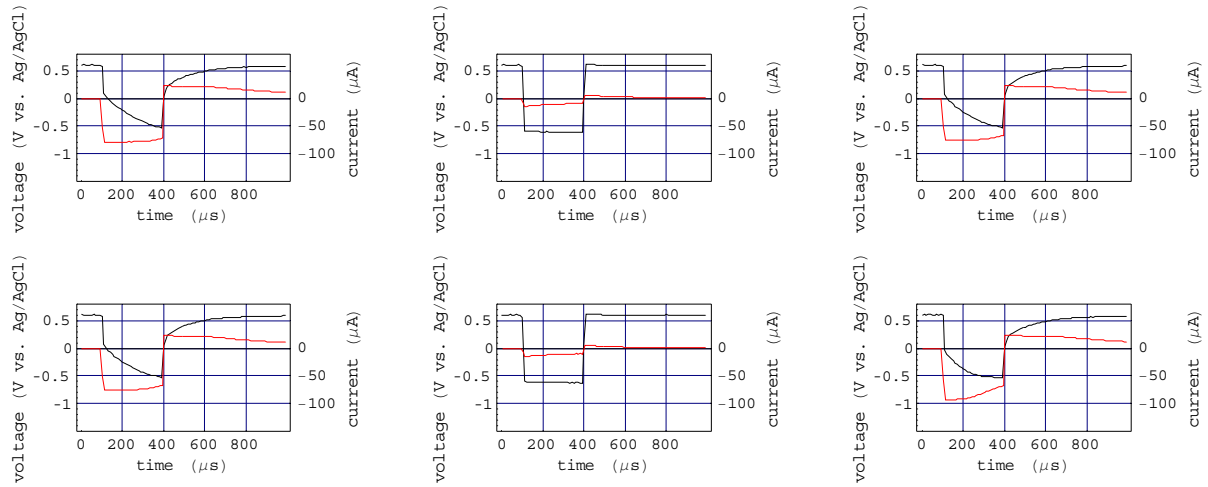

Fig. 2. Current pulse waveform of AIROF microelectrode *MPA08* (red trace: current; black trace: voltage). Plots on the first row are curent and voltage waveforms of *MPA08* when it is: pre-implantation in 1/16 PBS (left column), in the bird's brain (middle column), and explanted in 1/16 PBS (right column). Plots on the second row are the same electrode implanted back into the bird's brain for the second time immediately following its explantation. This repeating step confirms that the iridium oxide film of the electrode remains intact during the implantation.

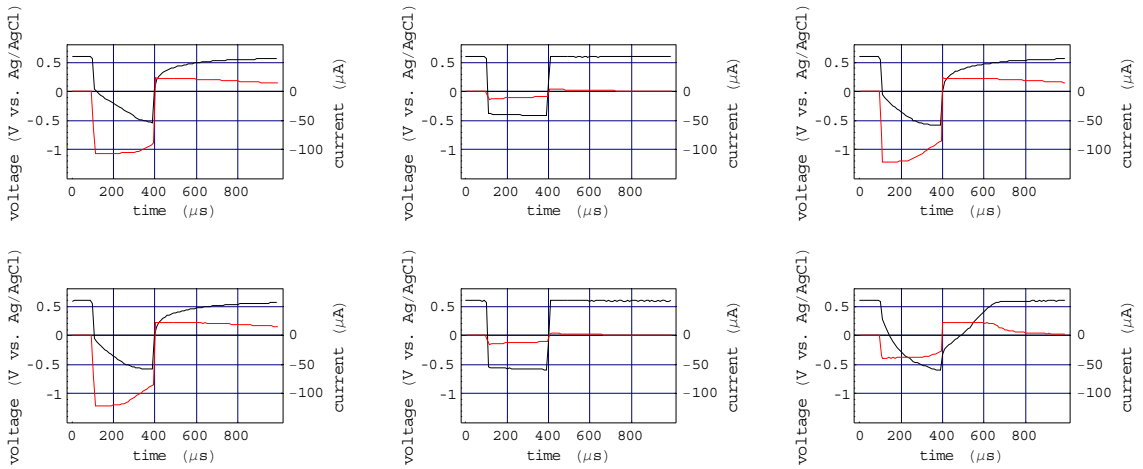

Fig. 3. Current pulse waveform of AIROF microelectrode *MPA06* (red trace: current; black trace: voltage). Plots on the first row are current and voltage waveforms of *MPA06* when it is: pre-implantation in 1/16 PBS (left column), in the bird's brain (middle column), and explanted in 1/16 PBS (right column). Plots on the second row are the same electrode implanted back into the bird's brain for the second time immediately following its explantation. During this second implant, due to some aggressive testing, the iridium oxide film was delaminated. So on the second explant, the electrode's charge capacity reduced (second row, third column).

Knowing the geometric surface area of the electrode and integrating the current waveform over 300 μs pulse width, we can estimate the charge density of the electrodes. For the chosen compliance limit, the *in vivo* value is about 10% of the *in vitro* ones for both electrodes.

#	in vitro (PBS)	in vitro (1/16 PBS)	in the bird brain
MPA06	1.69 mC/cm^2	1.33 mC/cm^2	0.14 mC/cm^2
MPA08	1.18 mC/cm^2	1.04 mC/cm^2	0.15 mC/cm^2

black (pre-implant) traces have similar characteristic reduction and oxidation current peaks of the iridium oxide film, which are evidence of the intact iridium oxide film during two repeated implantation and explantation procedures. On the other hand, on the first row, one brown trace (of the second explantation) shows collapsed CV loops and a metal reduction current tail (first row, middle column) in 50 mV/s CVs. This suggests that the iridium oxide film of the electrode was delaminated and the bare iridium metal was exposed.

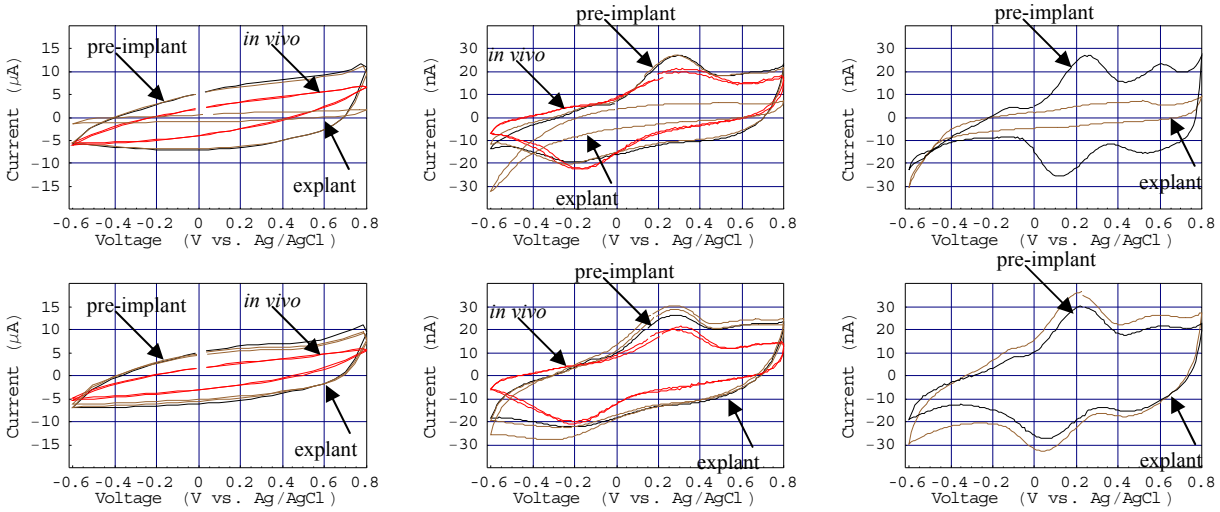

Fig. 4. CV measurements of *MPA06* (first row) and *MPA08* (second row). First column: 50 V/s scan rate (in 1/16 PBS); second column: 50 mV/s (in 1/16 PBS); third column: 50 mV/s (in PBS). Two red traces: electrode implanted in the bird's brain; two brown traces: electrode explanted; black trace: before implant. Plots on the first row, one brown trace collapses and has a metal reduction current tail (middle) in 50 mV/s CV. It is due to the iridium oxide damage during the aggressive testing in the second implant.

B. Cyclic Voltammetry

Fig. 4 shows the 50 mV/s and 50 V/s scan rate CV loops of both electrode *MPA06* and *MPA08* in 1/16 PBS as well as in PBS. On the second row, the brown (explant) and the

While the electrode was in the brain, the CV loops (red traces) are smaller, especially the 50 V/s fast CV, as compared with the in-vitro measurements. This is consistent with the smaller peak current in Fig. 2, reflecting the reduced charge capacity of the electrode *in vivo*.

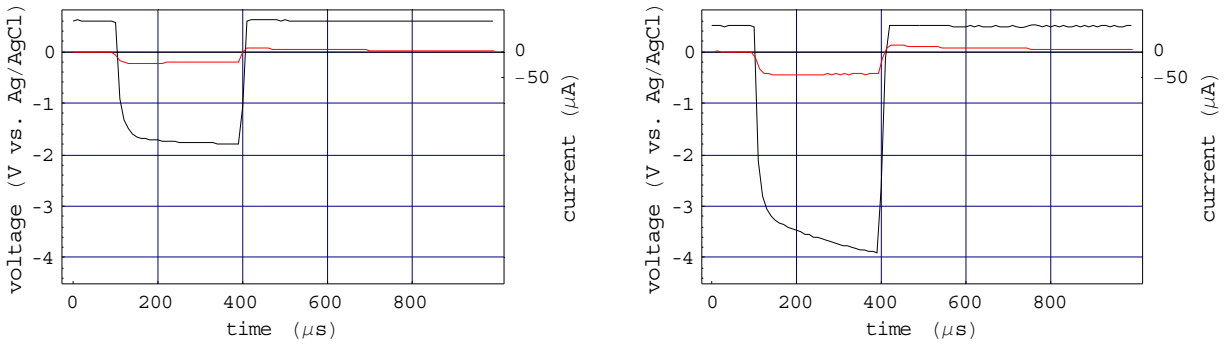

Fig. 5. Aggressive testing of *MPA06* in the bird brain. In the left graph, the negative compliance limit was allowed to drop to −1.8V. The estimated access resistance is *78.5 k*Ω; in the right graph, the negative compliance limit was further lowered to close to −4 V and the access resistance estimated is *71.4 k*Ω.

C. *Aggressive Testing*

To further test the limits of the *in vivo* charge delivery capacity of the AIROF microelectrode, we deliberately set the cathodic compliance limit more negative to allow for larger voltage excursion during the cathodic current phase.

Fig. 5 shows the pulsed current and voltage waveforms for electrode *MPA06* during its second implant. An "access resistance" can be estimated from the leading edge of the voltage waveform while knowing the current being delivered and is found to be on the order of 70 kΩ. As a means of attempting to compensate for this access resistance, the voltage excursion, during pulsing, was allowed to go more negative by the amount of the access resistance drop. For *MPA06,* we tested what voltage excursion would be required to support a stimulus current of 50 μA, while maintaining access resistance correction. In this case, the voltage reached approximately 4 V negative (vs. Ag|AgCl). Later, when *MPA06* was explanted and measured again *in vitro*, the collapsed CV loop was seen in the upper right-hand plot of Figure 4, evidently suggesting that the iridium oxide film had delaminated. This was further confirmed by our subsequent digital microscope image which showed bare iridium metal and no suggestion of AIROF. For *MPA08*, we did the similar aggressive testing and we limited the voltage excursion to −1.8 V. There is no evidence of iridium oxide film damage in the explant CV loop (Fig. 4. second row) and it was confirmed by the digital microscope image later.

IV. Conclusion & Discussion

In these experiments, both AIROF microelectrodes showed diminished (only 10%) charge delivery capacity *in vivo*. For compliance-supply limited driving, the behavior of the electrodes, *in vitro*, remained constant, before and after implantation, suggesting no damage to the electrodes. This suggests that the difference between the *in vitro* and *in vivo* charge capacity is caused by the relative nature of the *in vivo* electrolyte. From the current pulse measurements, the problem seems to be a large access resistance, although the cause is uncertain. Most likely polarization concentration is an important factor. Although often cited as a strategy, compensating for this access resistance by allowing the voltage excursion on the electrode to be more negative could result in irreversible damage to the film, as was seem for one of the electrodes. For all applications employing AIROF electrodes, it is important to limit the voltage excursions so as to prevent film damage. Although the consequences of damaging negative voltages on AIROF may be more immediately apparent than for Pt electrodes, this study does not suggest that Pt electrodes, with their inherently lower charge density capabilities are immune to voltage-induced damage at the electrode/tissue interface. For all metal electrodes, understanding the consequences of excessive voltage excursions is essential to maintaining integrity of implanted stimulating electrodes.

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