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In vivo and *In vitro* Differences in the Charge-injection and Electrochemical Properties of Iridium Oxide Electrodes

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Abstract—**The electrochemical response of activated iridium oxide (AIROF) electrodes implanted acutely in the subretinal space of the rabbit is compared with *in vitro* measurements in model electrolytes. Voltage transients during current pulsing, cyclic voltammetry, impedance spectroscopy and open-circuit potential measurements were compared. Subretinal charge injection by constant current pulsing required significantly greater driving voltages due to both higher access resistances (iR) and large electrochemical polarization across the electrode-tissue interface. Differences in the *in vivo* and *in vitro* open-circuit potentials were also noted. The data suggest that the buffering capacity in the subretinal space, at least in the acute study, is higher than that in inorganic models of interstitial fluid. Possible origins of these differences in terms of electrode-tissue interactions are discussed.**

I. Introduction

The electrochemical properties of low impedance electrodes used for neural stimulation and recording are typically characterized by cyclic voltammetrey, impedance spectroscopy and voltage transients during current pulsing. These methods are usually applied *in vitro* in an electrolyte that approximates the NaCl concentration and sometimes the buffering capacity of the interstitial and cerebrospinal fluids. However, the extracellular fluids are characterized by a much wider range of inorganic and organic constituents than a typical phosphate buffered saline (PBS) and, importantly, an inhomogeneous and constrained diffusional environment that limits the transport of reactive and charge-compensating species to or from the electrode. In the present paper, *in vitro* and *in vivo* electrochemical measurements from iridium oxide electrodes are compared. The observed differences between in the *in vivo* and *in vitro* environments are used to help develop a better understanding of charge-transfer at the electrode-tissue interface and identify limitations of electrode testing in unconstrained, large-volume liquid cells.

The electrochemical and charge-injection properties of electrodes are also compared in several model electrolytes that attempt to mimic, to varying degrees, the inorganic composition and buffering capacity of extracellular fluid. These measurements highlight the large differences in charge-injection capacity that are obtained with electrodes in model environments that have only modest compositional differences.

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II. Procedures

A. Animal Model

Electrochemical measurements were made with electrode arrays implanted acutely (<15 hr) in the subretinal space of the Dutch-belted rabbit. These studies were carried out at the Boston VA Hospital, Jamaica Plains Campus under institutional animal care and use committee approval.

B. Multielectrode Arrays

Multielectrode arrays developed for subretinal visual prostheses were provided through the Center for Innovative Vision Research, Boston VA Healthcare System, and fabricated at the Cornell Nanofabrication Laboratory. The arrays consisted of 15, 400-μm diameter, iridium electrodes in a 3 x 5 pattern on a ~10 μm thick polyimide substrate, as shown in Fig. 1. The iridium was activated by potential pulsing in 1N H_2SO_4 to form activated iridium oxide (AIROF) charge injection coatings. For these studies, the cathodic charge storage capacity of the AIROFs was typically 10 to 15 $mC \cdot cm^{-2}$. The proximal end of the electrode array was terminated in gold bonding pads that provided mechanical contact points for electrical connection to electronic instrumentation.

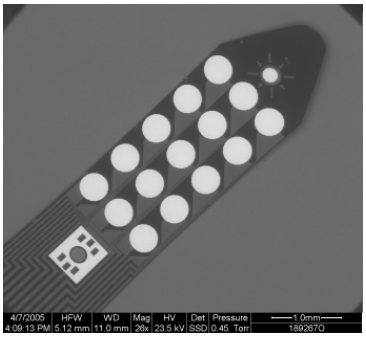

Fig. 1 Distal portion of a subretinal microelectrode array with 15 (3 x 5), 400 □m diameter stimulation electrodes.

C. Electrochemical Measurements

Open-circuit potential, cyclic voltammetry and electrochemical impedance measurements were made with Gamry potentiostats using vendor-supplied software. All *in vitro* measurements were made in a three-electrode cell using a large-area Pt counterelectrode, a Ag|AgCl reference electrode, and the electrode under study, with most measurements made at 37ºC. Three electrolyte models of extracellular fluid were employed: 1) PBS - 0.13M NaCl, 0.022M $NaH_2PO_4 \cdot H_2O$, 0.081M $Na_2HPO_4 \cdot 7H_2O$ at pH ~7.3; 2) CBS/PBS 137 mM NaCl, 29 mM $NaHCO_3$, 1.7 mM

Na_2HPO_4, and 0.7 mM NaH_2PO_4 purged with a 5% CO_2/6% O_2/89% N_2 gas mixture to pH 7; and 3) ISF - Na_2HPO_4-$7H_2O$ (2 mM), NaH_2PO_4-H_2O (0.5 mM), $NaHCO_3$ (28 mM), $KHCO_3$ (7.5 mM), NaCl (110 mM), $MgSO_4$ (0.5 mM), $MgCl_2$ (0.5 mM), $CaCl_2$ (0.5 mM) sparged with 5% O_2, 6% CO_2, 89% N_2.

Stimulation current pulsing studies were performed with a Sigenics stimulator that provides rectangular, cathodal current pulses. Both *in vivo* and *in vitro* pulsing was performed at a positive bias (V_b) of 0.6 V (vs. Ag|AgCl), a pulse width (PW) of 0.4 ms, and a frequency of 50 Hz [1]. Charge-balance during pulsing is achieved by re-establishing V_b after each pulse. The access voltage (V_{acc}), associated primarily with iR drops within the electrolyte (tissue), was taken as the initial voltage excursion at the onset of the current pulse and used to calculate the maximum negative potential excursion (E_{mc}) of the electrode by,

$$E_{mc} = V_b + (V_{drv} - V_{acc}) = V_b + E_{pol} \qquad (1)$$

where V_{drv} is the maximum driving voltage, noting that V_{drv} and V_{acc} are negative for a cathodal current pulse, and E_{pol} is the polarization across the electrode-electrolyte interface.

III. Results

Voltage transients recorded during current pulsing of a 400-μm diameter AIROF electrode *in vitro*, (in ISF) and after implantation subretinally in the rabbit are compared in Fig. 2 for 1 ms cathodal pulses from 0.1-0.8 mA. The *in vitro* and *in vivo* transient parameters are listed in Tables I and II, respectively. Included in Tables I and II is the apparent capacitance (C_{app}) at each current level, calculated from $C_{app} = Q_{inj} \cdot E_{pol}^{-1}$. Higher C_{app} values reflect the ability of an electrode to inject charge with less polarization and large values of C_{app} are generally desirable.

A comparison of E_{mc} and V_{drv} is provided in Fig. 3 as a function of current. From the E_{mc} values it is also clear that the electrochemical polarization across the electrode-electrolyte interface in the subretinal placement is considerably higher than that encountered in ISF, and the maximum injectable charge is considerably less *in vivo*. The V_{drv} in the present measurement is the sum of the polarization at the electrode-electrolyte interface and the voltage drop within the electrolyte or tissue close to the electrode. Neglecting minor voltage drops at the counter electrode or other iR-drops in the driving circuit, V_{drv} determines the power required to deliver a current pulse. The power required is about three times larger *in vivo* than *in vitro*.

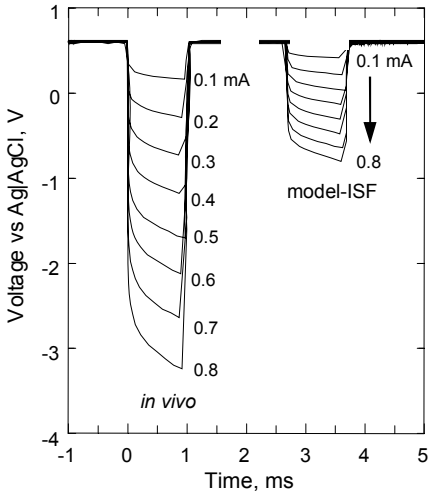

Fig. 2. Comparison of *in vivo* (subretinal) and *in vitro* (ISF) voltage transients of an AIROF electrode pulsed at currents from 0.1-0.8 mA. More severe access voltages and electrode polarization are encountered subretinally.

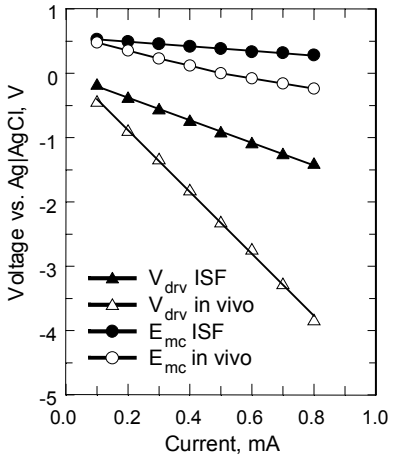

Fig. 3. Comparison of *in vivo* and *in vitro* driving voltage (V_{drv}) and cathodal potential excursion (E_{mc}) as a function of pulse current.

TABLE I
IN VITRO VOLTAGE TRANSIENTS, V_B=0.6 V, PW=0.4 ms

I A/cm^2	Q_{inj} μC•cm^{-2}	V_{acc} V	E_{mc} V	E_{pol} V	C_{app} μF•cm^{-2}
0.08	80	0.12	0.528	-0.06	1327
0.16	159	0.28	0.492	-0.104	1531
0.24	239	0.424	0.456	-0.14	1706
0.32	318	0.56	0.416	-0.18	1769
0.40	398	0.704	0.384	-0.216	1843
0.48	478	0.832	0.334	-0.258	1852
0.56	557	0.976	0.316	-0.284	1962
0.64	637	1.1	0.282	-0.31	2055

TABLE II
IN VIVO VOLTAGE TRANSIENTS, V_B=0.6 V, PW=0.4 ms

I A/cm^2	Q_{inj} μC•cm^{-2}	V_{acc} V	E_{mc} V	E_{pol} V	C_{app} μF•cm^{-2}
0.08	80	0.316	0.476	-0.128	622
0.16	159	0.64	0.352	-0.256	622
0.24	239	0.968	0.228	-0.372	642
0.32	318	1.32	0.12	-0.5	637
0.40	398	1.72	0.0	-0.6	663
0.48	478	2.06	-0.08	-0.68	703
0.56	557	2.52	-0.16	-0.76	733
0.64	637	3.0	-0.24	-0.84	758

Although the voltage transients during current pulsing indicate that charge-transfer *in vivo* requires a higher driving voltage and more severe electrode polarization, slow-sweep-rate (50 mV/s) cyclic voltammetry suggested more facile *in vivo* charge transfer. A comparison of *in vitro* (ISF and PBS)

and subretinal CVs of an AIROF electrode are shown in Fig. 5. The *in vivo* response was intermediate between that obtained in ISF and PBS. Electrodes evaluated by *in vitro* cyclic voltammetry after explanation recovered the pre-implantation *in vitro* response. This difference in the *in vitro* and *in vivo* CV response was observed for >30 electrodes in five subretinal implantation studies.

Differences in the *in vivo* and *in vitro* EIS response of an AIROF electrode are compared in Fig. 6. At high frequencies (>1 kHz) the impedance magnitude *in vivo* is higher than *in vitro*, which is consistent with the observation of a higher *in vivo* V_{acc}. At lower frequencies, the *in vivo* impedance magnitude is less than that observed *in vitro*, consistent with the more facile *in vivo* charge transfer suggested by the differences in CV response.

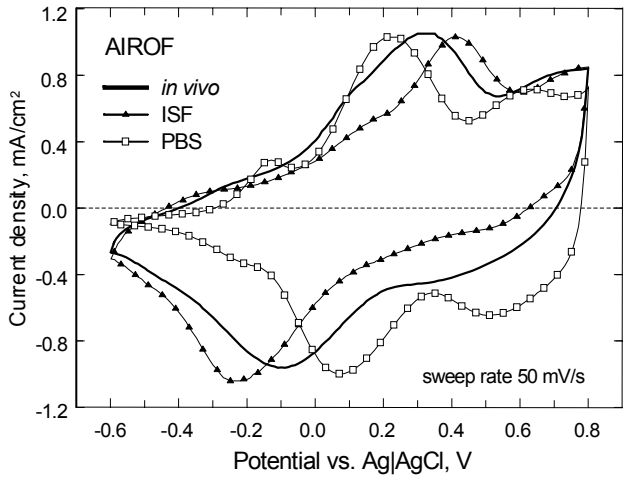

Fig. 5. CV comparison of an AIROF electrode taken subretinally (no symbols) and in ISF and PBS electrolyte models, reflecting the relative pH buffering capacity of the electrolytes models and the subretinal fluid.

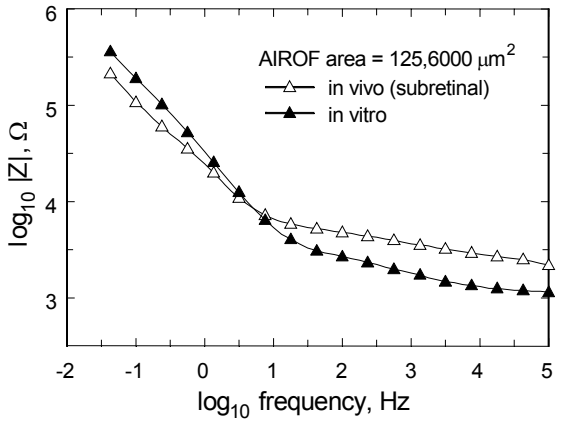

Fig. 6. Comparison of *in vivo* and *in vitro* (ISF) impednace magnitude of an AIROF microelectrode showing a higher, *in vivo* high-frequency impedance due to increased iR and a reduced lower frequency *in vivo* impedance relative to measurements made in ISF.

The charge-injection capacity of iridium oxide electrodes is increased significantly by pulsing the electrode from a positive potential bias. *In vitro*, the optimum bias is about 0.6 V vs. Ag|AgCl, although it is necessary to employ asymmetric current pulses or other strategies to limit the positive potential excursion during the charge balancing anodal phase to avoid water oxidation [2]. The current necessary to sustain a non-equilibrium electrode potential was compared *in vivo* and *in vitro* over a 0.0–0.7 V (Ag|AgCl) range. The *in vivo* currents were consistently higher than observed *in vitro*, requiring approximately twice the current density to sustain an optimal 0.6 V bias. In addition, the potential of zero current, corresponding to the open-circuit potential of the iridium oxide, was consistently more negative *in vivo*. Open-circuit potential measurements as a function of time following implantation showed a steady negative shift from about 0.2 V to 0.0 V vs. Ag|AgCl over the first hour of *in vivo* exposure. A compendium of V_{oc} values is provided in Table III. In this particular study, seven electrodes on the array were underneath coagulated blood from hemorrhage during surgery and these electrodes had a statistically significant V_{oc} from either the blood-free implanted electrodes or the pre-implantation V_{oc}'s in model ISF.

TABLE III
OPEN-CIRCUIT POTENTIALS OF IRIDIUM OXIDE ELECTRODES

CONDITION	MEAN ± S.D V VS. Ag|AgCl	NO.
Initial, model ISF	0.23 ± 0.06	15
Subretinal, no hemorrhage	-0.02 ± 0.01	8
Subretinal, under hemorrhage	0.10 ± 0.04	7
Explanted, model ISF	0.25 ± 0.01	15

IV. DISCUSSION

In the acute subretinal study, charge-injection is characterized by a higher V_{acc} and by a lower apparent capacitance. The higher *in vivo* V_{acc} reflects a higher ionic resistance in the tissue around the electrode compared with model ISF, which has an ionic conductivity of 10 $mS \cdot cm^{-1}$, intermediate between that of cerebrospinal fluid (18 $S \cdot cm^{-1}$) and that of gray matter (3.3 $mS \cdot cm^{-1}$) [3]. The access voltages estimated from the transients were closely linear with current (Fig. 3), and average subretinal and *in vitro* R_{acc} values of 3800 Ω and 1400 Ω, respectively, were calculated from a least square fit to the slopes in Fig. 3. Access resistance values ranged considerably in these acute studies, varying between 2 kΩ and 8 kΩ, but were always greater than *in vitro* R_{acc} measured in ISF. Higher access resistances necessitate higher driving voltages and place correspondingly greater demands on implanted electronic drivers and transcutaneous power transmission systems.

The difference in CV response of iridium oxide subretinally and in ISF suggests more effective pH buffering *in vivo* than in the inorganic interstitial fluid model. The potentials of the maximum current for the primary Ir^{3+}/Ir^{4+} reduction and oxidation waves are shifted to more negative and positive values, respectively, in ISF. In PBS, which has a high, non-physiological buffer concentration, the peak current potentials are shifted less than observed *in vivo*. Based on the pH dependence of -72 mV/pH-unit for the

AIROF V_{oc}, the local pH at the AIROF electrode could range from 4 to 10 during the *in vivo* CV sweep.

Interestingly, the large negative shift in V_{oc} (Table III) suggests a significantly alkaline subretinal pH, at least in the first 15 hr following implantation. A pH titration of the V_{oc} of an AIROF electrode is plotted in Fig. 7. Included in Fig. 7 is the average *in vivo* V_{oc}, which suggests a pH of ~9.4 in the subretinal space. However, the CV data from Fig. 5 and measurements of water oxidation and reduction potentials *in vivo* (data not shown) indicate a more neutral pH, suggesting that V_{oc} shift has other origins besides pH.

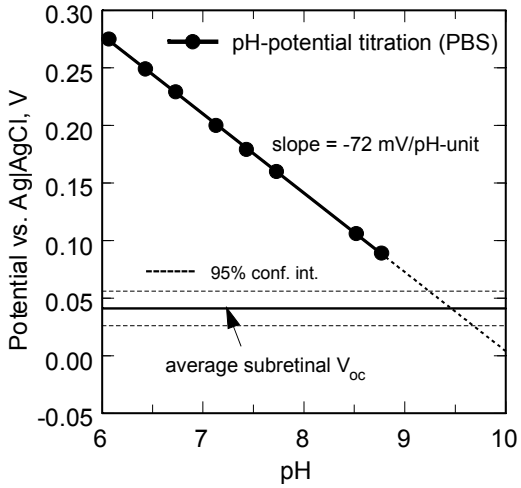

Fig. 7. Open-circuit potential of AIROF as a function of pH showing the average V_{oc} obtained after 1 hr or longer implantation subretinally (dashed lines = 95% confidence interval, 50 electrodes, 11 animals).

The lower C_{app} *in vivo* suggests that the charge injection is less facile *in vivo* than in the ISF model. Charge-injection limits depend on the electrolyte used for measurement as shown in Fig. 8, for AIROF microelectrodes in the three model electrolytes, ISF, CBS/PBS, and PBS. In ISF, Q_{inj} is notably lower than in either CBS/PBS or PBS. We have observed that Q_{inj} is not dependent on buffer concentration, but does show an apparent dependence on the ionic conductivity of the electrolyte even though the R_{acc} is corrected for in calculating Q_{inj}. We hypothesize that this dependence is due to differences in counterion availability during high-current-density, charge injection. Counterions are necessary for charge neutrality in the AIROF, or at any electrode interface, during charge-injection. If the rate of counterion transport to the electrode for a reversible process, e.g. reduction and oxidation of the Ir^{3+}/Ir^{4+}, is limited during constant-current pulsing, other reactions must be recruited to support the current. Typically, these reactions would be reduction or oxidation of water, which results in a lower Q_{inj}, if these irreversible processes are to be avoided.

Since the *in vivo* environment of an electrode is diffusionally constrained, it likely that the reversible *in vivo* Q_{inj} will be less than that measured *in vitro*. The *a priori* application of *in vitro* charge-injection limits to implanted electrodes may therefore pose a greater than anticipated challenge to the electrode. This raises the question of how

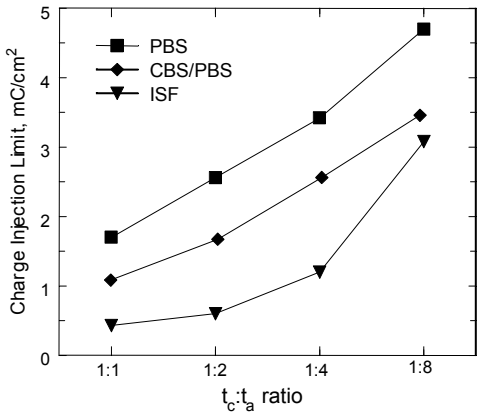

Fig. 8. Charge-injection capacity as a function of current waveform asymmetry using biphasic current pulses in three different electrolyte models of extracellular fluid.

to safely deliver charge *in vivo* and what the criteria for safe stimulation, with respect to the electrode, should be.

V. Conclusion

Differences in the *in vivo* and *in vitro* electrochemical response and charge-injection capabilities of AIROF electrodes implanted subretinally in rabbit have been evaluated. Not surprisingly, the *in vivo* behavior of the electrodes was different from that obtained with inorganic models of extracellular fluid. *In vivo* charge-transfer reactions at the AIROF electrodes appear to be more facile at low current densities (CV response) and slower at high current densities (pulse response) than observed in an inorganic ISF model. The *in vivo* environment also presents diffusional limitations on charge transfer that is not well modeled by liquid electrolytes and this may result in lower *in vivo* Q_{inj} limits than those measured *in vitro*. The principal limitation of the present study is the acute nature of the *in vivo* measurements, which should now be extended to chronic studies.

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