Protocol: Surface Modification of Microelectrode Arrays

Description: This document details the surface modification protocol for polymer microelectrode arrays (pMEAs) to enhance the electrochemical performance of microelectrodes. The current protocols are designated for Parylene MEAs but could be adapted for MEAs made using polyimide. In this procedure, electrodeposition of platinum-iridium (PtIr), poly(3,4-ethylenedioxythiophene) polystyrene sulfonate (PEDOT:PSS) coatings, and iridium-oxide (IrOx) onto electrode sites are performed via cyclic voltammetry (CV).

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1 pMEA and PCB Preparation

1.1 Requirements

Materials: *Packaged pMEA*

1. pMEAs should be bonded to PCBs as described in Parylene MEA Packaging Protocol.

1.2 Evaluation of Device

Materials: *Packaged pMEA*
1× PBS
Platinum counter electrode
Male-to-female Omnetics connector
Equipment: *LCR meter*

1. Set LCR meter to measure impedance using a 1 kHz, 25 mV signal
2. Connect male-to-female Omnetics connectors to the connector attached to the PCB
3. Submerge shanks in 1× PBS. Do not submerge PCB
4. Place the platinum counter electrode in 1× PBS
5. Connect the counter electrode to one of the LCR meter probes
6. Probe each electrode by connecting the other LCR meter probe to each Omnetics connector wire
    a. If impedance magnitude is greater than 3 MΩ, the electrode is broken.
    b. If more than 10% of the electrodes are broken, the pMEA should not be used
7. Remove all electrodes, gently dip electrodes in DI water three times, and allow to air dry

2 Inspect and Test

2.1 Visual Inspection

Materials: *Packaged pMEA*
Equipment: *Optical microscope*

1. Carefully place pMEA with exposed electrodes facing up (for up-right microscope) on the microscope stage
2. Collect images using 5×, 10×, and 20× magnification of important features of the pMEAs
    a. Examine metal traces for faulty connections
    b. Examine electrode openings for remaining Parylene. If electrodes look contaminated or with remaining polymer, re-examine after CV in H_2SO_4.

2.2 Cyclic Voltammetry (H_2SO_4)

Materials: *Packaged pMEA*
Ag/AgCl reference electrode
Platinum counter electrode
0.05 M H_2SO_4
Equipment: *Potentiostat with faraday cage*

1. Submerge the electrode end of the shanks in DI water for 1-5 minutes
2. Prepare a 50 mL beaker with ~25 mL of 0.05 M H_2SO_4 and purge with N_2
    a. Purge for at least 5 minutes prior to beginning CV
    b. Continue purging during testing if possible
3. Remove the device from the DI water and submerge the shanks of the device in the H_2SO_4; do not submerge deep enough such that the connection and PCB are submerged
4. Rinse the Ag/AgCl reference electrode and platinum counter electrode in DI water, then place them in the H_2SO_4 beaker, taking care not to touch the pMEA
5. Connect the reference electrode, counter electrode, and one lead wire (working electrode) to the potentiostat in a 3-electrode setup
6. Perform CV for 30 cycles from -0.2 to 1.2 V with 250 mV/s scan rate
    a. Calculate the electroactive surface area and cathodic charge storage capacity using the equations in appendix A and appendix B
7. Move the working electrode connection to other wires and repeat
8. Turn off N_2 purging
9. Remove the reference and counter electrodes from beaker and rinse with DI water
10. Remove the pMEA from the beaker and resubmerge in DI water three times
11. If not proceeding to the next test within a day, remove the device from DI water and allow to air dry

2.3 Electrochemical Impedance Spectroscopy (PBS)

Materials: *Packaged pMEA*
50 mL beaker
Ag/AgCl reference electrode
Platinum counter electrode
PBS
Equipment: *Potentiostat with faraday cage*

1. If the pMEA is dry, submerge shanks in DI water for 1-5 minutes
2. Prepare 50 mL beaker with ~25 mL of 1× PBS
3. Remove pMEA from DI water and submerge shanks in PBS; do submerge deep enough such that the connection/PCB are submerged
4. Rinse the Ag/AgCl reference electrode and platinum counter electrode in DI water, then place them in the PBS beaker, taking care not to touch the pMEA
5. Connect the reference electrode, counter electrode, and one lead wire (working electrode) to the potentiostat in a 3-electrode setup
6. Perform EIS using 25 mV_{rms} over the range of 1 to 10^5 Hz
7. Move the working electrode connection to other wires and repeat
8. To test the insulation integrity of the lead connection point, add more PBS to the beaker until the lead connection is submerged and repeat EIS – any significant changes in impedance and phase indicate leakage in the connection point
9. Proceed to Voltage Transient Measurement in PBS

2.4 Cyclic Voltammetry (PBS)

Materials: *Packaged pMEA*

Ag/AgCl reference electrode
Platinum counter electrode
1x PBS

Equipment: *Potentiostat with faraday cage*

1. Submerge the electrode end of the shanks in DI water for 1-5 minutes
2. Prepare a 50 mL beaker with ~25 mL of 1× PBS and purge with N_2
    a. Purge for at least 5 minutes prior to beginning CV
    b. Continue purging during testing if possible
3. Remove the device from the DI water and submerge the shanks of the device in 1× PBS; do not submerge deep enough such that the connection and PCB are submerged
4. Rinse the Ag/AgCl reference electrode and platinum counter electrode in DI water, then place them in the 1× PBS beaker, taking care not to touch the pMEA
5. Connect the reference electrode, counter electrode, and one lead wire (working electrode) to the potentiostat in a 3-electrode setup
6. Perform CV for 3 cycles from -0.6 to 0.6 V with 200 mV/s scan rate. The reason for -0.6 V to 0.6 V is because this range is within the water window limits of Pt, PtIr, IrOx and PEDOT:PSS.
    a. Calculate the electroactive surface area and cathodic charge storage capacity using the equations in appendix A and appendix B
7. Move the working electrode connection to other wires and repeat
8. Turn off N_2 purging
9. Remove the reference and counter electrodes from beaker and rinse with DI water
10. Remove the pMEA from the beaker and resubmerge in DI water x3
11. If not proceeding to the next test within a day, remove the device from DI water and allow to air dry

2.5 Voltage Transient Measurement (PBS)

Materials: *Packaged pMEA*
50 mL beaker
Ag/AgCl reference electrode
Platinum counter electrode
PBS

Equipment: *Potentiostat with faraday cage*

1. Maintain the same electrode configuration as in EIS
2. Stimulate one pulse with 0.05 µA amplitude, 200 µs pulse width, and 100 µs interpulse delay and record resulting voltage
3. Calculate the interphase potential from the resulting voltage pulse
    a. Continue pulsing until the current to reach approximately -0.6 V interphase potential is reached
    b. Obtain at least three data points with the interphase potential greater than -0.6 V (i.e., a smaller negative value)

    Note: if the interphase potential is smaller than -0.6 V (i.e., a larger negative value), decrease the current amplitude
4. Move the working electrode connection to other wires and repeat
5. Remove the reference and counter electrodes from beaker and rinse with DI water
6. Disconnect the pMEA and submerge shanks in DI water three times

7. Allow the pMEA to dry in air
8. Calculate the charge injection capacity (CIC) for each electrode using the equations in app appendix C

3 Surface Modification

This protocol includes surface modification via electrodeposition of PtIr, PEDOT:PSS, and IrOx. The selection of which material to use depends on long-term performance and accessibility. Long-term performance will be evaluated, and results disclosed. PEDOT:PSS and IrOx coatings can be performed in-house whereas PtIr is outsourced to an external company.

3.1 Platinum-Iridium (PtIr)

Note: PtIr coatings are performed by EPIC Medical, Inc. Contact Artin Petrossians, Ph.D. (artinpetros@gmail.com) for further inquiries.

3.2 Poly(3,4-ethylenedioxythiophene) Polystyrene Sulfonate (PEDOT:PSS)

Materials: *Packaged pMEA*
50 mL beaker
Ag/AgCl reference electrode
Platinum counter electrode
Poly (sodium 4-styrenesulfonate)
3,4-ethylene dioxythiophene (EDOT)
Equipment: *Potentiostat with faraday cage*
Note: solution preparation is explained in depth in ref. [1]

1. Prepare a 20 mL solution of the PEDOT 0.1 %/PSS 0.8% mixed with DI water
    a. Remove air/gas from the solution by bubbling with N_2 for 20 minutes prior to electrochemical experiments
2. Submerge shanks in PEDOT:PSS solution; do not submerge deep enough such that the connection/PCB are submerged. Short all wires to deposit PEDOT:PSS on all electrodes
3. Place reference and counter electrode in the solution
4. Connect the reference electrode, counter electrode, and the shorted wires to the potentiostat in a 3-electrode setup
5. Perform CV for 3 cycles from -1.0 to 1.0 V with 10 mV/s scan rate
6. Remove the reference and counter electrodes from beaker and rinse with DI water
7. Remove the pMEA from the beaker and submerge in DI water three times
8. Allow the pMEA to dry in air

3.3 Iridium Oxide (IrOx)

Materials: *Packaged pMEA*
50 mL beaker
Ag/AgCl reference electrode
Platinum counter electrode
2.0 mM potassium hexachloroiridate (III) (K_2IrCl_6)
10 wt% of aqueous sodium hydroxide (NaOH)

3 M nitric acid (HNO_3)
NaOH
Equipment: Potentiostat with faraday cage
Note: solution preparation is explained in depth in ref. [2]

1. Prepare electrodeposition solution
    a. Adjust 2.0 mM aqueous K_2IrCl_6 to pH 13 with 10 wt% of aqueous NaOH – should result in a yellow solution
    b. Heat solution to 90 °C for 20 minutes
    c. Cool down solution to room temperature and transfer to ice bath until obtaining a blue solution
    d. Adjust the cold solution to pH 1 by rapidly adding 3 M HNO_3
    e. Stir continuously for 80 minutes until the solution becomes deep blue
    f. If not using the solution right away, store in a refrigerator at 2 °C
2. Submerge shanks in the solution; do submerge deep enough such that the connection/PCB are submerged. Short all wires to deposit IrOx on all electrodes
3. Place reference and counter electrode in the solution
4. Connect the reference electrode, counter electrode, and the shorted wires to the potentiostat in a 3-electrode setup
5. Perform CV for 100 cycles from 0.2 to 0.75 V with 50 mV/s scan rate
6. Remove the reference and counter electrodes from beaker and rinse with DI water
7. Remove the pMEA from the beaker and submerge in DI water three times
8. Allow the pMEA to dry in air

4 INSPECT AND TEST

4.1 pMEAS NOT USED FOR *IN VIVO* STUDIES

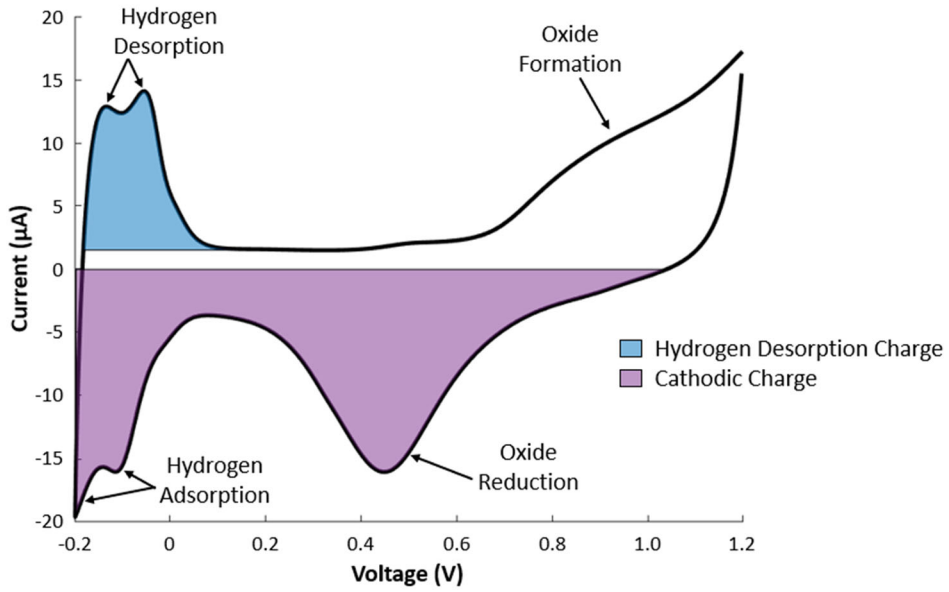
Note: skip if the device will be used for implantation
Repeat steps from section 2 to evaluate and compare the performance of the coated and non-coated pMEAs

4.2 pMEAS USED FOR *IN VIVO* STUDIES

Repeat steps from section 1.2 to evaluate the impedance at 1 kHz

APPENDICES

A. SURFACE AREA CALCULATION (H_2SO_4)

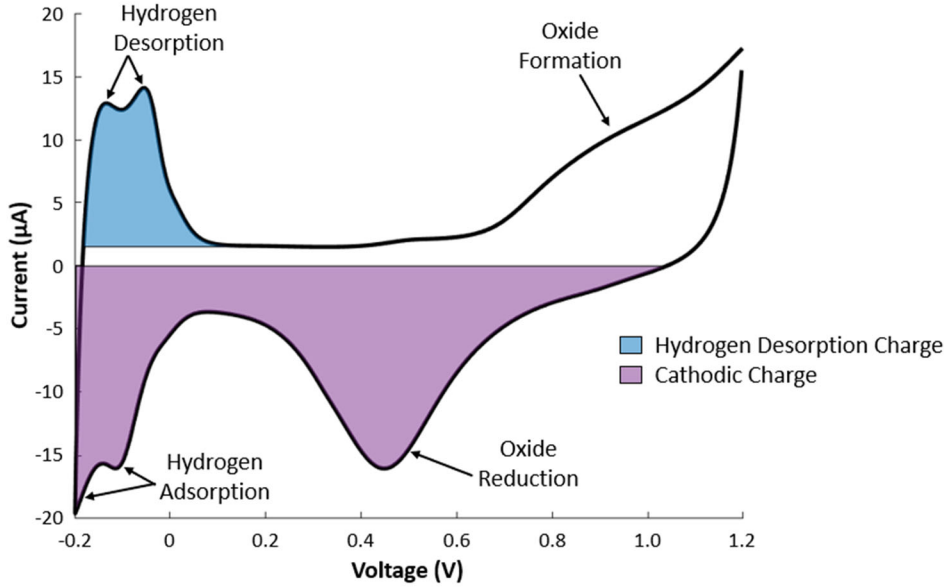


$$Electrochemical\ Surface\ Area\ (ESA) = \frac{Q_H}{\rho_H}$$

$$Hydrogen\ Desorption\ Charge\ (Q_H) = time\ integral\ of\ hydrogen\ desorption\ current$$

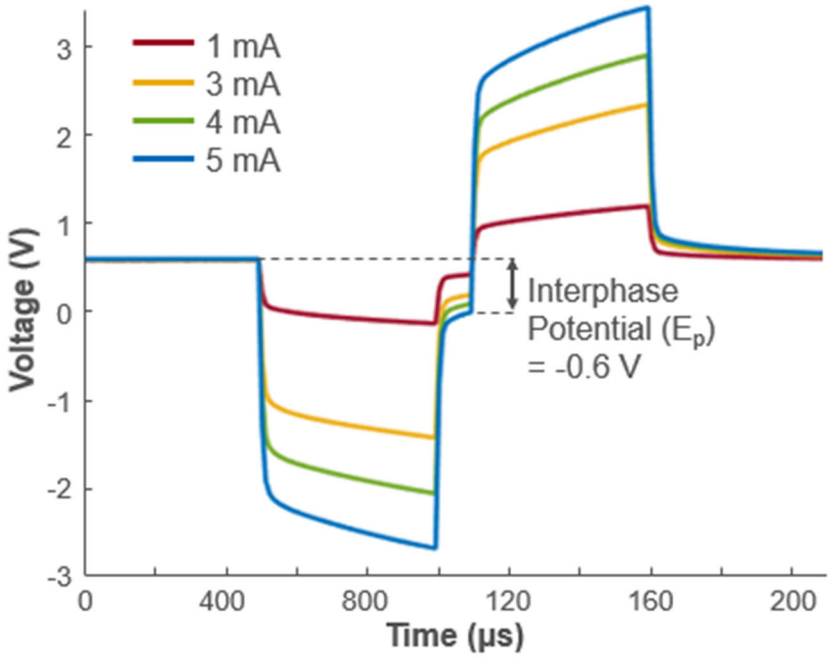
$$Characteristic\ charge\ density\ of\ monolayer\ of\ hydrogen\ atoms\ adsorbed\ to\ polycrystalline\ Pt\ (\rho_H) = 210\frac{\mu C}{cm^2}$$

B. CATHODIC CHARGE STORAGE CAPACITY CALCULATION



$$Charge\ Storage\ Capacity\ (CSC) = \frac{Q_{cathodic}}{GSA}$$
$$Cathodic\ Charge\ (Q_{cathodic}) = time\ integral\ of\ cathodic\ current$$
$$Geometric\ Surface\ Area\ (GSA) = geometric\ area\ of\ electrode$$

C. Charge Injection Capacity Calculation



$$Charge\ Injection\ Capacity\ (CIC) = \frac{(current\ @E_p = -0.6) \times (pulse\ width)}{GSA}$$

$$Geometric\ Surface\ Area\ (GSA) = geometric\ area\ of\ electrode$$

D. Material Sources

Note: Standard materials (e.g. acetone, DI water, cleanroom wipes, etc.) are not listed

Material	**Supplier**
Nanostrip 2X	CMC Materials, Santa Ana, CA
Ag/AgCl (3M) reference electrode	Basi Research Products, West Lafayette, IN
3,4-ethylene dioxythiophene (EDOT)	Sigma Aldrich, St. Louis, MO
Poly (sodium 4-styrenesulfonate) (NaPSS, average Mw70,000)	Sigma Aldrich, St. Louis, MO
2.0 mM potassium hexachloroiridate (III) (K_2IrCl_6)	Sigma Aldrich, St. Louis, MO
Nitric acid (HNO_3, 70%)	Sigma Aldrich, St. Louis, MO
Sodium hydroxide (NaOH)	Fisher Scientific

E. Equipment Models

Note: Standard equipment (e.g. tweezers, microscopes, N_2 gun, scale, etc.) are not listed

Equipment	Model #	Supplier
LCR Meter		Agilent, Santa Clara, CA
Potentiostat Galvanostat	Reference 600	Gamry Instruments Inc., Warminster, PA

F. REFERENCES

1. V. Castagnola, E. Descamps, A. Lecestre, L. Dahan, J. Remaud, L.G. Nowak, and C. Bergaud, "Parylene-based flexible neural probes with PEDOT coated surface for brain stimulation and recording," *Biosen. Bioelec.*, vol. 67, p. 450-457, 2015, doi: 10.1016/j.bios.2014.09.004.
2. M. Khalil, S. Wang, J. Yu, R.L. Lee, and N.Liu, "Electrodeposition of Iridium Oxide Nanoparticles for pH Sensing Electrodes," *J. Electrochemical Soc.*, vol. 163, no. 9, B485-B490, 2016, doi: 10.1149/2.0391609jes.