Spring 2015

Biofabrication and Evaluation, in vitro and in vivo, of a Dual Responsive Glucose and Lactate Implantable Biosensor in a Piglet Trauma Model

Olukayode Karunwi
Clemson University

Fouzan Alam
Clemson University

Anthony Guiseppi-Elie
Clemson University

Biofabrication and Evaluation, *in vitro* and *in vivo*, of a Dual Responsive Glucose and Lactate Implantable Biosensor in a Piglet Trauma Model

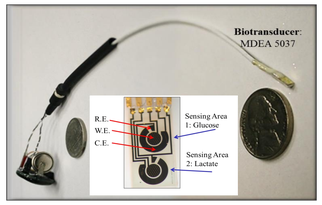
Olukayode Karunwi[1,2] (Graduate), Fouzan Alam[1,3] Melissa Gaillard[1,2] and Anthony Guiseppi-Elie[1,2,3,4]

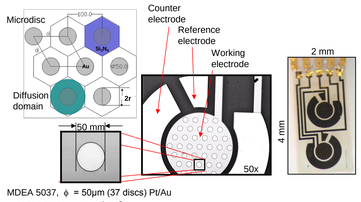
[1]Center for Bioelectronics, Biosensors and Biochips (C3B), Departments of [2]Bioengineering, [3]Chemical and Biomolecular Engineering, and [4]Electrical and Computer Engineering, Advanced Materials Research Center, Clemson University, Anderson, SC 29625

Tel: +1 (309) 830-1953, Email: okarunw@clemson.edu; Tel: +1 (864) 656 1712, Email: guiseppi@clemson.edu

1. Introduction

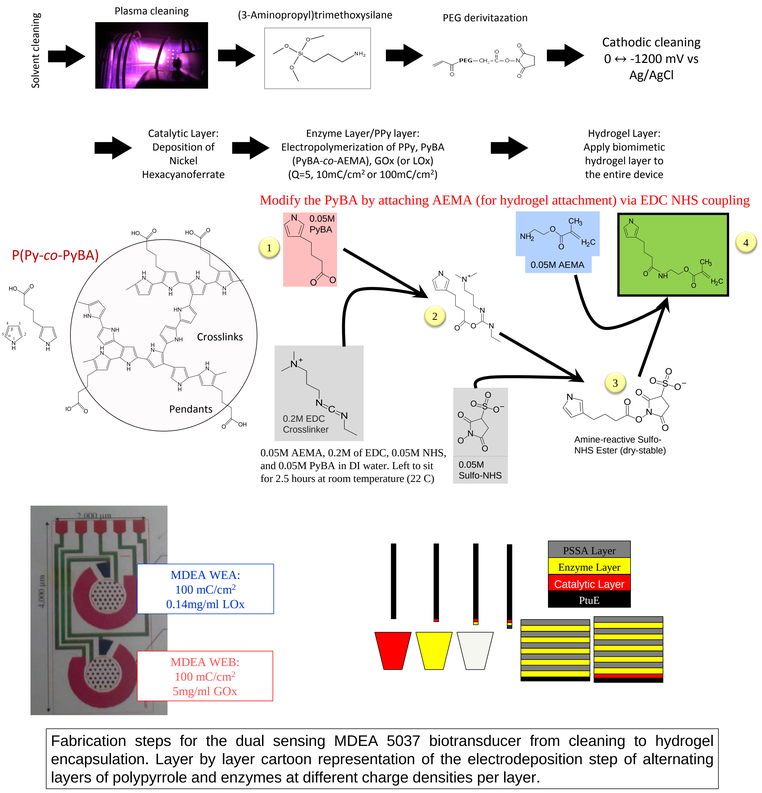
- Pressing need for the design and development of a device for **simultaneous** measurement of interstitial levels of glucose and lactate levels during **hemorrhage**
- Minimally invasive, dual responsive Electrochemical Cell-on-a-Chip Microdisc Electrode Array (ECC MDEA 5037) has been developed for use with a wireless biosensor system.
- Fabrication of the biorecognition membrane via pyrrole electropolymerization, ***in vitro*** and ***in vivo*** characterization of the resulting biotransducer (as well as the intermediate steps in fabrication) are described.



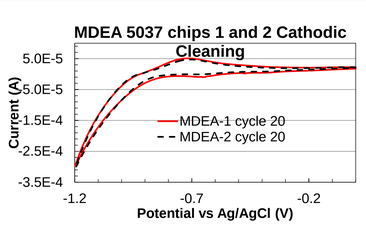


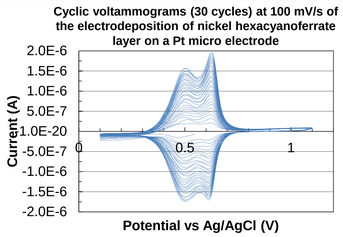
- A dual responsive Electrochemical Cell-on-a-Chip Microdisc Electrode Array (ECC MDEA 5037) powered by a wireless potentiostat and developed for use in a wireless implantable biosensor system. Insert shows the reference electrode (R.E.), working electrode (W.E.) and counter electrode (C.E.) for each electrochemical cell area
- Front-end details of the MDEA5037 device

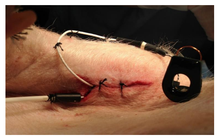
2. Materials and Methods

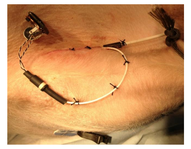


Fabrication steps for the dual sensing MDEA 5037 biotransducer from cleaning to hydrogel encapsulation. Layer by layer cartoon representation of the electrodeposition step of alternating layers of polypyrrole and enzymes at different charge densities per layer.



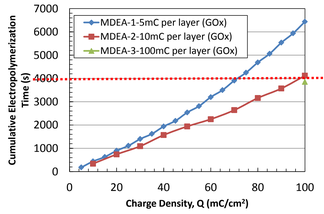


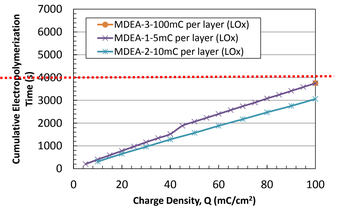




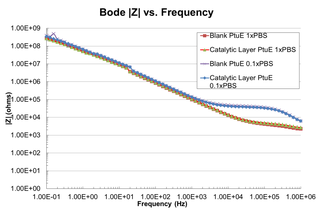
- Sample data from cathodic cleaning after 20 cycles on 2 different MDEA devices. Demonstrates consistency and reproducibility of electrode areas
- Cyclic voltammograms (30 cycles) at 100 mV/s of the electrodeposition of nickel hexacyanoferrate layer on a Pt micro electrode
- Implantation of dual (glucose and lactate) sensing device in shoulder muscle of piglet

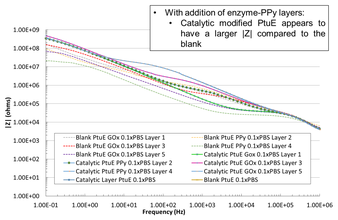
3. Results and Discussion

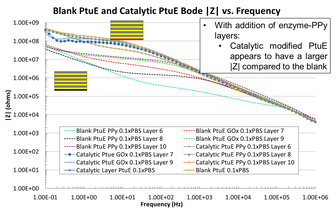


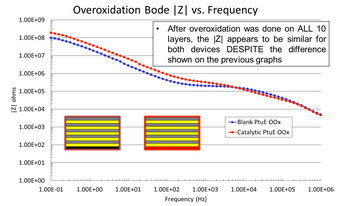


- Cumulative electropolymerization time vs. cumulative charge density for glucose oxidase (GOx) and lactate oxidase (LOx) systems at different charge densities

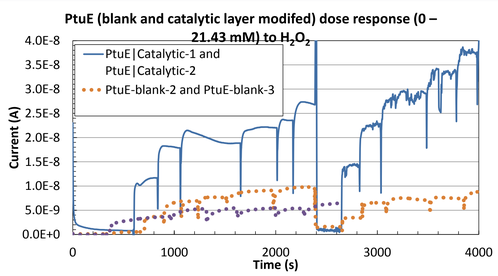


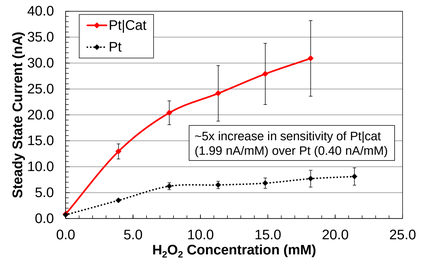




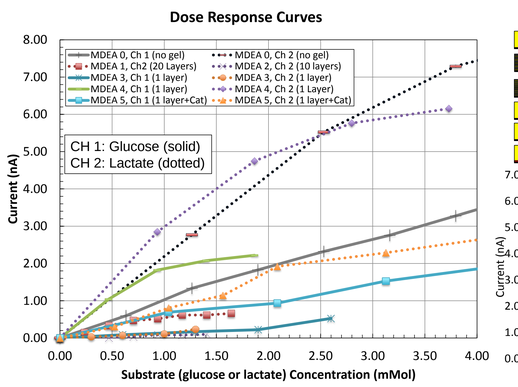


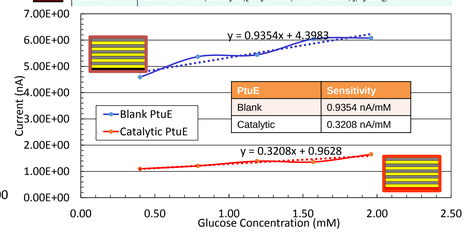
- The variation in the sets of curves is due to the difference in buffer conditions
- **Both** the blank and catalytic layer modified devices appear **similar** and do not show any variation in |Z|





Role of catalytic layer: Blank sensor vs. catalytic sensor response to varying H_2O_2 concentration





Sensor	Official Name
MDEA 0	MDEA 5037-Pt|[PPy-GOx(100mC cm-2)]
MDEA 1	MDEA-Pt|[PPy-GOx(10mC cm-2) |PPy-PSSA(10mC cm-2)] x5|hydrogel
MDEA 2	MDEA-Pt|[PPy-GOx(5mC cm-2) |PPy-PSSA(5mC cm-2)] x10|hydrogel
MDEA 3	MDEA 5037-Pt|[PPy-GOx(100mC cm-2)]|hydrogel
MDEA 4	MDEA 5037-Pt|[PPy-GOx(100mC cm-2)]|hydrogel
MDEA 5	MDEA 5037-Pt|Catalyst |[PPy-GOx(100mC cm-2)]|hydrogel

Device	Sensitivity (nA/mM) - Glucose	Sensitivity (nA/mM) - Lactate	Response Time
MDEA 5037-Pt|[PPy-GOx(100mC cm^{-2})]	2.19	1.05	12 minutes (no hydrogel layer)
MDEA-Pt|[PPy-GOx(10mC cm^{-2}) |PPy-PSSA(10mC cm^{-2})]5|gel	N/A	0.70	18-20 minutes
MDEA-Pt|[PPy-GOx(5mC cm^{-2}) |PPy-PSSA(5mC cm^{-2})]10|gel	N/A	0.07	18-20 minutes
MDEA 5037-Pt|[PPy-GOx(100mC cm^{-2})]|gel	3.05	1.95	10-14 minutes (thinner hydrogel layer)
MDEA 5037-Pt|**Catalyst**|[PPy-GOx(100mC cm^{-2})]|gel	0.67	0.57	10-14 minutes (thinner hydrogel layer)

- Response to glucose and lactate concentrations in the tissue of the piglets and device details
- *In vitro* characterization: Comparing role of catalytic layer
- *In vivo* characterization: Summary of sensitivities and response times for various implanted devices.

4. Conclusions and Summary

- ❑ Demonstrated dual glucose and lactate responsive biotransducer
- ❑ Increasing the charge density does not appear to change the biotransduction characteristics significantly
- ❑ The catalytic modified devices showed consistency in electrodeposition
- ❑ Electrochemical Impedance Spectroscopy (EIS)
  - The catalytic layer did not change the impedance of the Pt electrode
  - The EIS data showed that each successive layer reduced the impedance of the blank device compare to the catalytic layer modified device
  - The overoxidation data showed that the impedance of both devices are similar regardless of catalytic layer
- ❑ *In vitro* dose response to glucose, showed that the blank device has a higher sensitivity (0.94 nA/mM) than the catalytic modified device (0.32 nA/mM)
- ❑ *In vivo* response to glucose and lactate showed that the device with **no catalytic** layer had ~5x (3.05 nA/mM *vs.* 0.67 nA/mM) the sensitivity to glucose and ~4x (1.95 nA/mM *vs.* 0.57 nA/mM) the sensitivity to lactate
  - The hydrogel coated device showed a greater sensitivity to glucose and lactate over the non hydrogel coated device

5. Key References

Karunwi, O., A. Wilson, C. Kotanen, and A. Guiseppi-Elie, *Engineering the abio-bio interface to enable more than Moore in functional bioelectronics*. Journal of The Electrochemical Society, 2013. 160(4): p. B60-B65.

Kotanen, C.N., O. Karunwi, and A. Guiseppi-Elie, *Biofabrication Using Pyrrole Electropolymerization for the Immobilization of Glucose Oxidase and Lactate Oxidase on Implanted Microfabricated Biotransducers*. Bioengineering, 2014. 1(1): p. 85-110.

Kotanen, C.N., F.G. Moussy, S. Carrara, and A. Guiseppi-Elie, *Implantable enzyme amperometric biosensors*. Biosensors and Bioelectronics, 2012. 35(1): p. 14-26.

Supported by the DoD PRMRP PR 023081 Grant Number DAMD 17-03-1-0172 and the C3B Consortium