Multi-Channel Potentiostate Integrated with Microfluidic Channel for Electrochemical Real-Time Monitoring

Yusra M Obeidat[a], Elaine Carnevale[b] , Adam J. Chicco[b,c] , Thomas W.Chen[a,c]

[a] Dept. of Electrical and Computer Engineering, [b] Dept. of Chemical & Biological Engineering, [c] School of Biomedical Engineering, Colorado State University

ABSTRACT

This work describes the development of an integrated sensors system to measure concentrations of dissolved oxygen (DO), pH, glucose, and lactate concurrently at single cell level. DO was measured amperometrically using a three-electrode system of working (WE), counter (CE) and reference (RE) electrodes. pH was measured potentiometrically using two electrodes system of Indium Tin Oxide (ITO) WE and Ag/AgCl RE. Glucose and lactate were measured enzymatically by measuring the current generated from the oxidation of hydrogen peroxide generated from the catalysis of glucose or lactate at the WEs with their catalysis enzymes. A microfluidic chamber containing all four sensors was made using SU8 to investigate single oocytes/embryos immersed in up to 120 µL of respiration buffer. This work includes the results of using the integrated sensors system to measure the metabolic activities of real cells including single oocytes or embryos. The micro-chamber was completely sealed using top layer of ovoil and covered by top glass lid to avoid oxygen exchange between the inside of the chamber and the atmosphere, while being maintained at a temperature of 38.5 ºC to preserve cell viability. The oxygen consumption of cells, the lactate production and glucose consumptions were measured as a change in output current and converted to femto-mol (fmol) per second based on calibrations with buffer of known DO, lactate, and lactate concentrations. This integrated sensor system has some potential applications include evaluating effects of metabolic therapies on oocyte bioenergetics, study the effect of aging on embryos development and monitoring mitochondrial function throughout oocyte maturation and blastocyst development to predict embryo viability to compliment assisted reproductive technologies.

BACKGROUND

- The metabolism of the cell is an important indicator for its health and activity, and it helps to determine its population and the disease that might affect its functionality.
- The most common metabolites of interest at the single cell level include oxygen, carbon dioxide, glucose, lactate. Combined with pH, they are good indicators of cellular metabolic activity.
- Electrochemical sensing methods have been successfully used for monitoring cellular activity.

ELECTRODES AND CHANNEL DESIGN

- Masks designed using AutoCAD

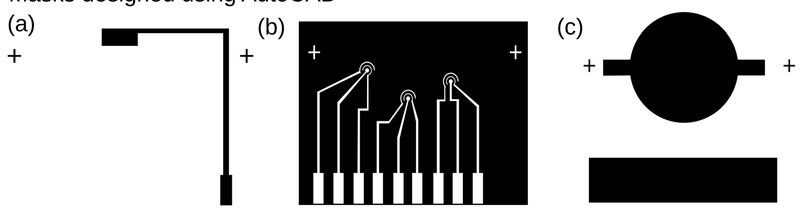

Figure 1: Masks used for the design(a) pH electrode (b) integrated gold electrodes for Glucose, Lactate and DO sensing (c) microfluidic channel

- Electrodes and microfluidic channel

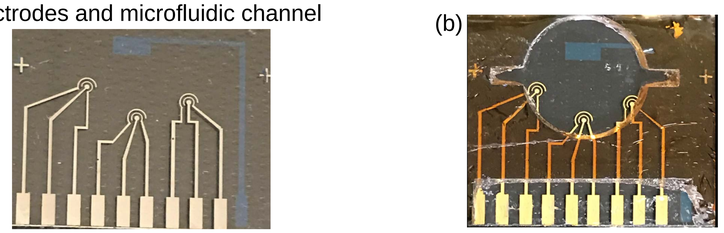

Figure 2: (a) complete electrodesdesign including: ITO pH electrode and integrated gold electrodes for Glucose, Lactate and DO sensing (b) microfluidic channel integrated with electrodes.

METHODS

- The sensor device was designed using photolithography and metal evaporation process.
- The device consists of multiple measurement sites. Each site is responsible for measuring a specific analyte using 3-electrode electrochemical cell configuration.
- Electrodes for measuring oxygen, glucose, and lactate are gold electrodes, and the electrodes for measuring pH are ITO electrodes.
- All electrodes are housed in a microchamber with a microfluidic channel of 1mm thick.
- DO, glucose and lactate were measured using an Amperometric method, while pH was measured using potentiometric method.
- Bovine oocytes and embryos were obtained by CSU's Equine Reproduction Laboratory, and matured in standard CDM-M buffer.

ELECTRODES FUNCTIONING

- **Cyclic Voltammetry results**

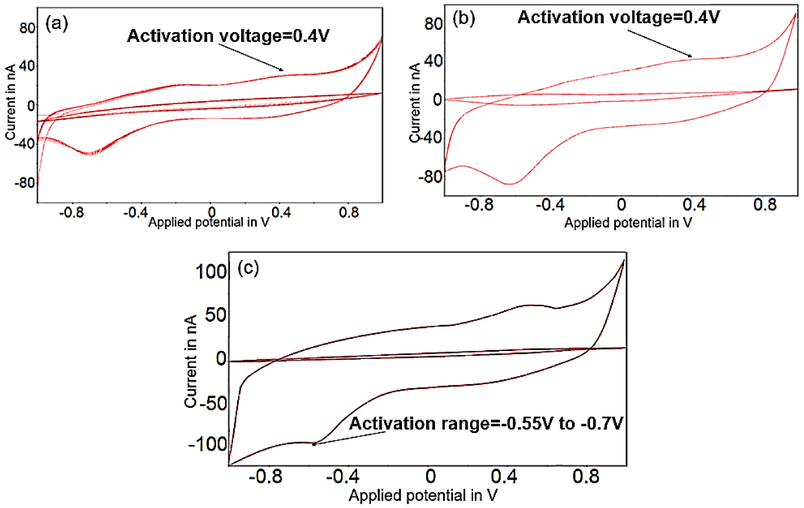

Figure 3: (a) CV results for Glucose sensor (b). CV results for Lactate sensor. (c) CV results for oxygen sensor.

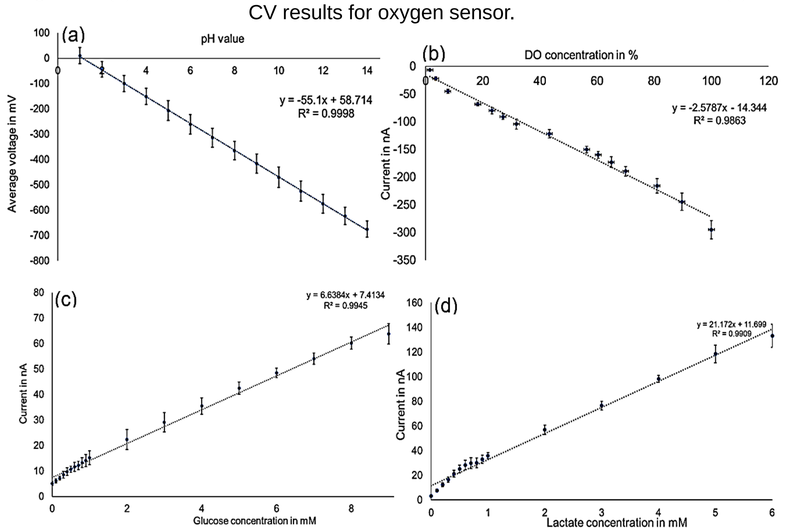

Figure 4: Calibration curves(a) pH sensor (b) Oxygen sensor (c) Glucose (d) Lactate (error bars in all figures are SD between 6 different tests)

RESULTS

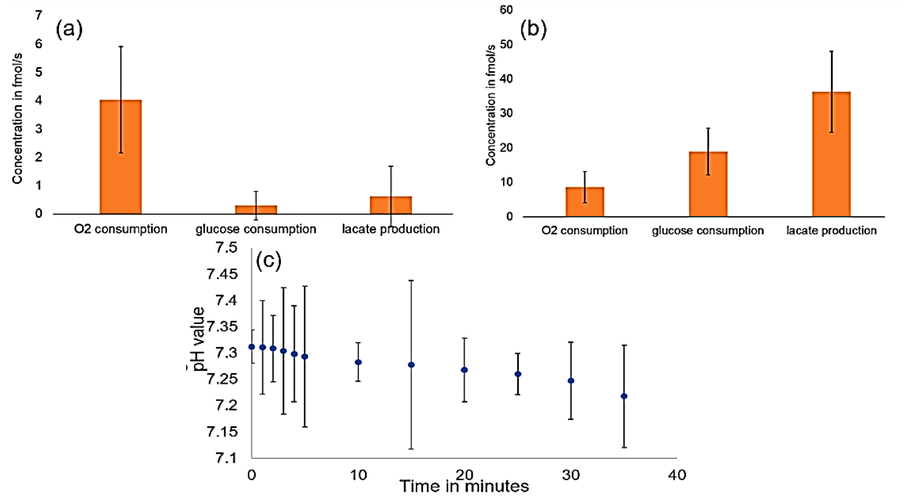

Figure 3: (a) Oxygen consumption, glucose consumption and lactate production for bovine oocytes (b) Oxygen consumption, glucose consumption and lactate production for bovine embryos, (c) measured pH change vs. time during metabolism of bovine embryos

CONCLUSION TABLE

Table of comparison between previous and this work

Cell type		O_2 range in fmol/s	Glucose range in fmol/s	Lactate range in fmol/s
Oocyte	Previous work	5.23-6.21	NA	NA
	This work	4±1.87	0.29±0.5	0.63±1.06
COC	previous	13.3-25	6.5-11.5	14.8-17.5
	this	NA	NA	NA
Embryo	previous	6.5-34	6.9-17.3	17-24.2
	this	8.5±4.5	18.9±6.7	36.3±11.8

CONCLUSIONS

A novel way has been developed to measure metabolic activity of cells. The integrated design with microfluidic channel is capable to concurrently measure DO, glucose, lactate and pH changes of the cell during its metabolism. This system has some potential applications include evaluating effects of metabolic therapies on oocyte bioenergetics, study the effect of aging on embryos development and monitoring mitochondrial function throughout oocyte maturation and blastocyst development .

REFERENCES

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[2] Yotter et al. "Sensor Technologies for Monitoring Metabolic Activity in Single Cells-Part II: Nonoptical Methods and Applications", IEEE sensors journal. VOL. 4, NO. 4, August 2004.