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Development of Oxygen Sensor by Integrating the Low Cost Printed Circuit Board Technology and Solid Electrolyte Membrane

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Abstract **-** This paper presents the development of a miniaturized Clark type oxygen sensor integrated with an in vitro cell culturing platform for the purpose of instantaneous monitoring of cellular oxygen consumption by the solution or gas flowing through the cultured cells on the platform. The cell culturing platform's prototype, which contains an inlet and outlet pipes and a cell culturing chamber, is being manufactured by The Eden250™ 3D Printing System using the Objet biocompatible material MED610™. The presented oxygen sensor configuration consists of two identical series of working, reference and counter microelectrodes, placed before and after the cell culturing chamber. It was manufactured by combining low cost printed circuit board technology and laser micro machining techniques, and was coated with a solid polymer electrolyte membrane, Nafion (perfluorosulfunic acid membrane, Du Pont Company) to ensure robustness and good electrical conductivity. The sensor can function easily without humidification or any special condition and has a long shelf life. The sensitivity of the oxygen sensor, having less than 3 seconds response time is tested in different oxygen concentration in gas state and was found to be compatible with measurements from a Portable Multi-Gas Analyzer provided by Super Systems Europe.

Keywords**:** Clark type oxygen sensor, Printed circuit board, Solid electrolyte, Instantaneous measurement.

1. Introduction

Oxygen is an important regulator of normal cell behaviour. It is one of the most important indicators of biological activity during cell culture and microbial development. Theoretically, the concentration of oxygen in a solution after it has passed through a chamber containing live cells will demonstrate key statuses of those cells, since the living cells will consume oxygen from solution and thus reduce its concentration versus before it passed through the chamber. The damaged cells will consume a lower amount of oxygen due to their decreased metabolic activities; dead cells won't consume any oxygen so there won't be any reduction in the oxygen concentration. Potential practical applications of the integrated oxygen monitoring device include pharmacological product testing. By utilizing two simultaneously operating oxygen sensors, the effects on the metabolism of the cells can be monitored continuously by reading the oxygen level of the solution or gas under test before and after adding the drugs and toxins to the cells. A compact and flexible testing environment with inexpensive disposable parts, increases efficiency due to lower fluid volumes, short diffusion distances, amongst other benefits.

The Clark type sensor is extensively used among the diverse instruments to measure oxygen in many clinical researches, fermentation monitoring and biosensor developments (Wilson, 2002). It was invented by Leland C. Clark in 1956 to detect low levels of oxygen (Clark, 1956).

As an electrochemical sensor, it has a low deviation among the sensors and produces good repeatability and reproducibility. In the past two decades there have been various developments on the Clark type sensors which can now be fabricated utilizing MEMS technology. Miniaturized dissolved oxygen sensors have been frequently produced using silicon-based and poly dimethyl siloxane (PDMS) materials for their ease of applying micro fabrication processes such as lithography, spincoating and wet/dry-etching on them (Park et al., 2013; Lee, Park, 2011; McLaughlin et al., 2002; Wu et al,. 2005).

However It has been proven to be difficult to incorporate these miniaturized sensors in the nature for their issues of robustness and short shelf life due to their need for rehydration, sophisticated sealing systems to prevent leakage of liquid electrolyte and continuous maintenance (Park et al., 2013; Lee, Park, 2011; Wu et al., 2005).Therefore an integrated cell culturing platform as a prototype was developed utilizing a 3D printing device, which can produce complex features inside the chip with the potential for cells to grow and adhere to its chamber.

Usage of a solid electrolyte eliminates the need of rehydration while increasing the shelf life, as described by Glen W. McLaughlin and co-workers (McLaughlin et al., 2002). Solid state proton conductive matrix (PCM) as an electrolyte encapsulated in a bio inert polytetrafluoroethelene (PTFE) has improved the performance of the microfabricated thin film electrode matrix and shown to have a linear response over 0 – 300 mmHg of dissolved oxygen concentration through cyclic voltammetry (CV) and voltage step (VS) measurements (McLaughlin et al., 2002).

Recently printed circuit board technology has created a potential as a MEMS platform for developing microsensors due to their advantages of robustness, acknowledged commercial production methods and good connectivity to standard systems (Cheneler et al., 2011). A miniature Clark sensor is easily manufactured on a printed circuit board as a sensor platform with a precision of 100 microns, and later micro machined with a laser micromachining device to achieve 20 microns dimension. "Having the advantages of robustness, easy connectivity to standard electrical systems and firm commercial fabrication techniques, PCB (Printed circuit board) makes it easy to fabricate microelectrode features like pads and tracks with gold coating and strong connectivity, which is difficult to achieve in silicon wafer processing and standard MEMS techniques" (Cheneler et al., 2011).

2. Clark Type Sensor's Principle of Operation

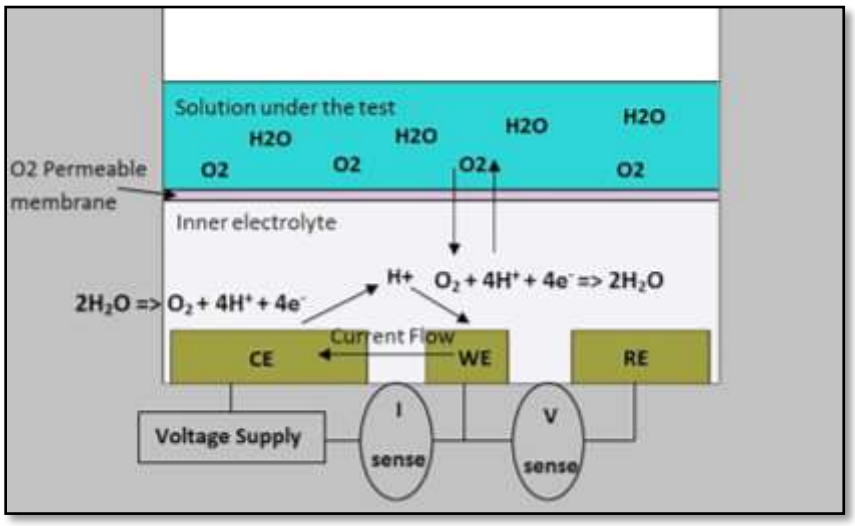


Fig. 1. Principle of oxygen sensor operation [redrawn from McLaughlin et al., 2005]

A Clark-type sensor consists of three electrodes, working (WE), reference (RE) and counter (CE). Electrolyte and an oxygen permeable membrane are overlaid on the electrodes, and usually a chamber is located on top of the permeable membrane to contain the solution under test. When the oxygen in the sample solution permeates through the membrane to the inner electrolyte, the reduction current of oxygen is measured at the WE as shown in Figure 1.

The dissolved oxygen is brought to the surface of WE and reduced electrochemically considering the equation (1) (Maruyama et al., 1998):

$$O_2 + 4H^+ + 4e^- => 2H_2O \quad (1)$$

According to equation (2) the resultant passes through the electrolyte to the CE and oxidizes.

$$2H_2O => O_2 + 4H^+ + 4e^- \quad (2)$$

The relation between the oxygen partial concentration, C, and the reduction current of oxygen can be shown by equation (3). Where F is faraday's constant, A is the surface area of the working electrode, n is the number of electrons and Pm is the permeability of the membrane.

$$I(t) = n\,F\,A\,Pm\,(\partial C/\partial x)_{X=0} \quad (3)$$

3. Design and Fabrication

Utilising 3D printing technology to create the cell culturing platform offers design flexibility and increases the number of design options versus conventional silicon manufacture techniques. The inlet and outlet pipes (with inner diameter of 1 mm) using a barbed fitting are designed to optimise and simplify the tube connection point and decrease the number of ports where leaks can occur and also reduce number of components and assembly time, whilst maintaining a strong seal. A U shaped bend was designed, where the fluid or gas is passing through the sensors and its dissolved oxygen is tested. The oxygen sensor configuration consists of two identical series of a WE, RE and CE microelectrodes, placed before and after the cell culturing chamber in order to detect the reduction of oxygen concentration in the fluid after being consumed by cells when they are cultured in the chamber.

Table 1. Dimensions and area ratios of the WE, RE and counter electrode.

Sensor	WE Diameter	Area ratio of WE:RE:CE	Gap size between electrodes	Commercially fabricated	Further laser machining applied
A	300 μm	1:5:25	100 μm	✓	
B	500 μm	1:2:6	100 μm	✓	
C	600 μm	1:1.5:3	100 μm	✓	
D	20 μm	1:5:25	20 μm	✓	✓

A printed circuit board that contains gold plated pad geometry to explore Clark Sensor O2 sensing techniques was designed and produced. Using Diptrace software, a PCB layout of two sensors on a 20 mm x 40 mm chip size was designed, with different diameter for WE and different area ratios of WE, RE and CE respectively as shown on Table (1). It was fabricated commercially by Wurth Elektronics with the minimum precision of 100 microns as it can be seen in Figure (2).

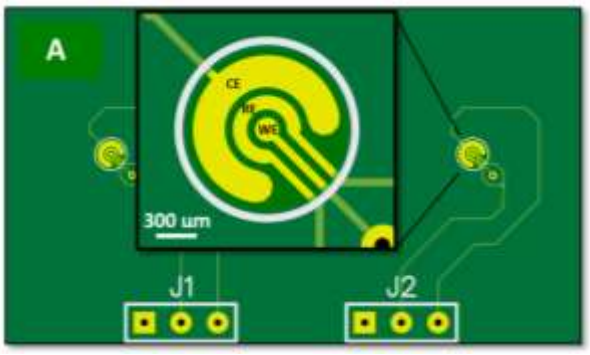


Fig. 2. Sensor's design with the minimum precision of 100 microns.

Further laser micro machining was done using a Lasea Multi-Axis laser micro machining station to reduce the size of working electrode to 20 microns as shown on Table (1) and its design on Figure (3). A double layer PCB of 1.55 mm thickness, and copper thickness of 35 microns with Nickel/Gold (4-7 µm Ni/0.05-0.1 µm Au) surface finish is chosen for this design.

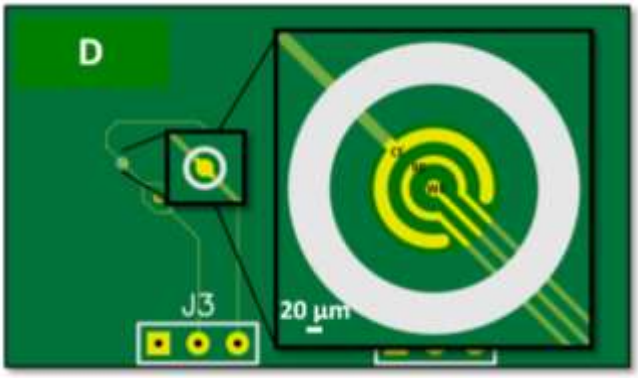


Fig. 3. Design of the sensor with 20 microns precision.

Nafion 117 membrane (perfluorosulfunic acid membrane, Du Pont Company) was used as a solid electrolyte with 183 microns thickness and 360 (g/m2) basis weights. First small area of the membrane (slightly bigger than the outer diameter of the CE) was cleaned by immersing in 60◦C deionised water for one hour and dried in room temperature. Then membrane hot pressed to the top of the electrodes on the printed circuit board in the oven for 2 hours in 120◦C, which were found to be the optimum duration and temperature for higher sensitivity and faster response time in the sensor. The PCB sensor was cleaned with deionised water previously. A thin laminated PTFE (polytetrafluorethylene) oxygen permeable membrane was applied on top of the Nafion, to limit diffusion of other species. After placing the O' rings to seal the cell culturing platform to the PCB in the specified location (Figure 4), two platforms are fastened and tightened to each other with four screws as shown in Figure (4). O' rings seal the sensors from the outside environment and from each other.

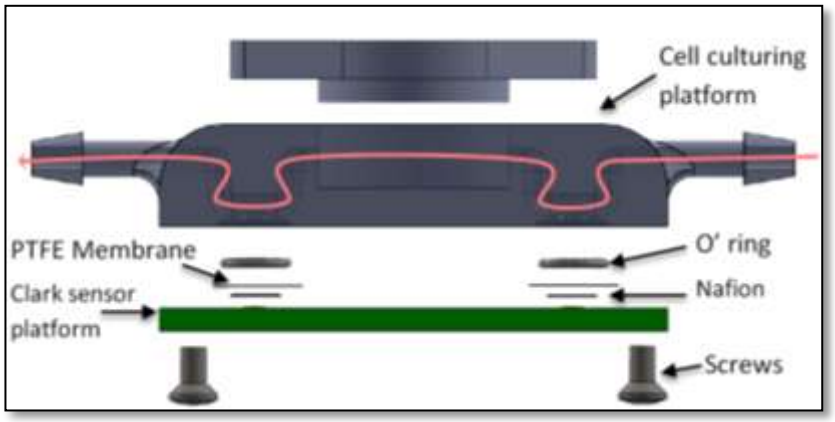
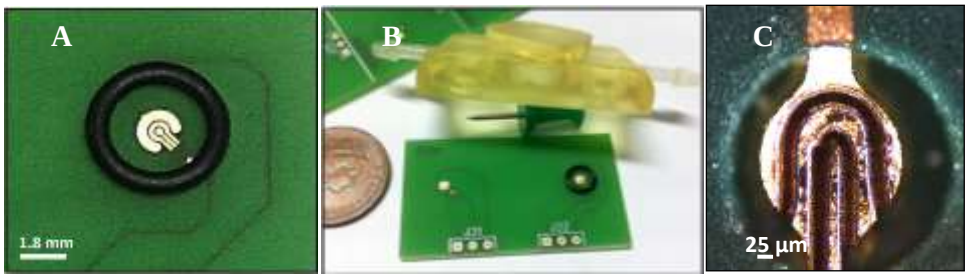


Fig. 4. Assembling the sensor platform and the cell culturing platform with Nafion electrolyte, PTFE membrane and O' rings.

Figure (5) shows the fabricated miniature Clark sensor and further micro machined electrodes and 3D printed cell culturing platform.



Fig, 5. A. The Ni/Au coated geometry of the Clark sensor fabricated on PCB. B. The primary cell culturing platform and the sensor platform is ready to assemble. C. Image of the 20 µm WE after laser micro machining, captured by Alicona G4 Infinite Focus system.

An analogue circuitry was designed to condition the low level signals to a point where they can be measured using DAQ (Data Acquisition) modules such as Labjack. The system initially runs from a dual PP3 9V battery to eliminate the possible noise issues. The input control is in the 0-5V range that is mapped to provide a 0-2V swing on the working electrode. The voltage sense provides a high impedance sense input to the reference electrode and has a gain of x2. The current sense will be able to detect the currents in Nano Ampere range.

99.999% nitrogen gas from a calibration gas cylinder with pressure regulators has been mixed with ambient air having 20% oxygen concentration and directed to a Portable Gas Analyser 3510 (PGA) to verify the concentration of the output gas from PGA [Figure 6]. By controlling the flow of the nitrogen, the gas in 0%-20% oxygen range is achievable in the output of the PGA. The gas from the output of the PGA is then directed to the oxygen sensor platform to test its functionality.

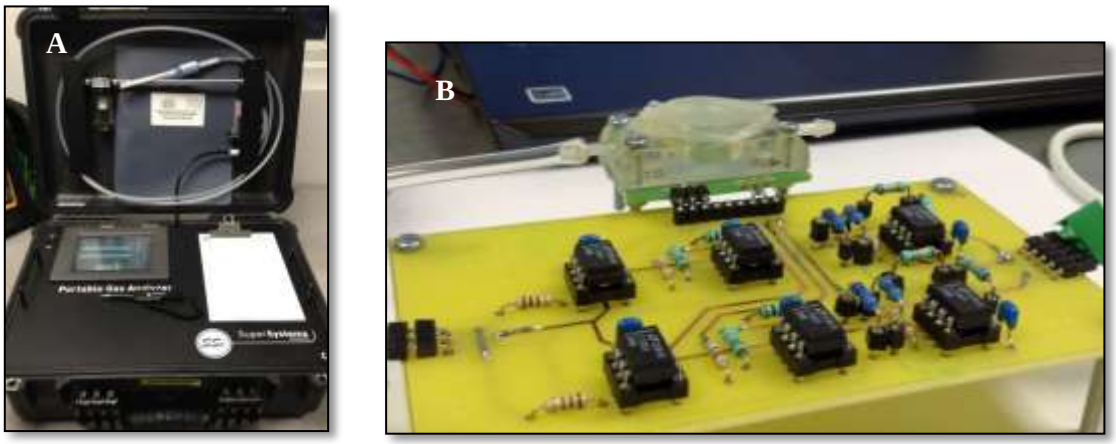


Fig. 6. A. Portable Gas Analyser 3510. B. The measurement circuit board with oxygen sensor platform

4. Results

4.1 Sensor Characteristics

To understand the behavior of the sensors and to find the applied sensing voltage, the I-V characteristic of the sensors were examined. As various bias potentials applied to the working electrodes of the sensors from -1 to 1 V, at certain bias potential between 0.8 and 1V current leveled off, this is the diffusion controlled region. The bias potential was set to 0.9 V and used for measurement of the oxygen concentration. The sensitivity of the oxygen sensor, having less than 3 seconds response time is tested in oxygen concentration in 0%-20% range and was found to be compatible with measurements from a Portable Multi-Gas Analyzer provided by Super Systems Europe.

4.2 Step Response Linearity

Step response linearity measurement was made on the sensors with four different sizes at oxygen concentration from 0 to 20% according to Figure (7). These measurements were made 3s after stabilization of the oxygen concentration reading from Portable Multi-Gas Analyzer after application of the step input. The sensors seem to have a consistent response and there is an almost linear relationship between the current and the oxygen concentration. The sensors with bigger WE diameters seem to measure higher amount of current since it has a bigger WE area, in line with equation 3.

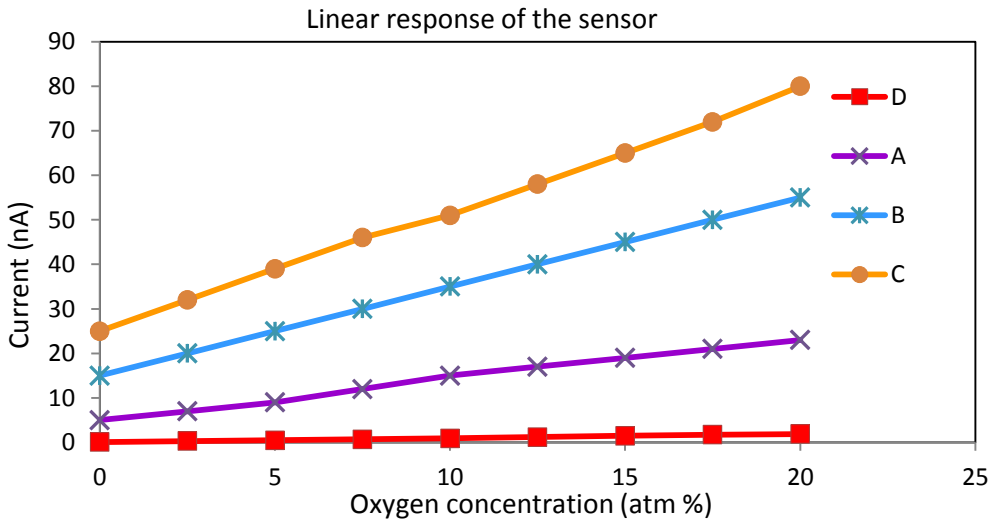


Fig. 7. Step linearity response of the WE having four different sizes between 0% to 20% oxygen concentrations.

4.3 Reproducibility of the Sensor

At 0.9V biasing potential applied to the WE, the real time response of the sensor has been measured in the sensor with the WE having diameter of 300 µm while reducing the oxygen concentration to 0% and increasing to 20%, 8 times as shown in Figure (8). The sensors showed fast response time, less than 3 seconds.

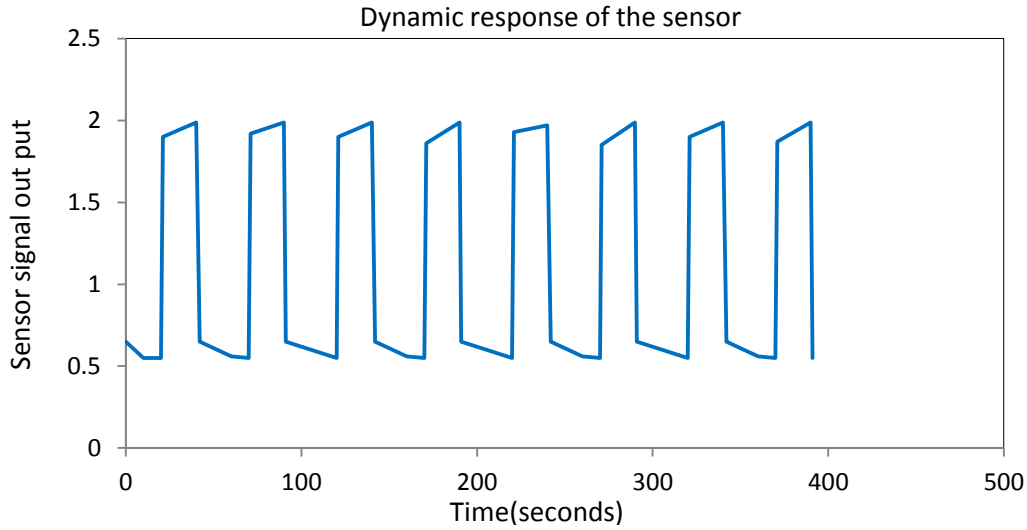


Fig. 8. Dynamic response of the WE having diameter of 300 µm between 0% to 20% oxygen concentration.

5. Conclusion

A 3D prototyped cell culturing platform integrated with a PCB based miniaturized Clark type oxygen sensors is presented. Considering four different sizes of the WE diameter with different ratio to their RE and CE, the result of the step response linearity and dynamic response are shown while exposed to oxygen concentration from 0% to 20%. They are shown to be highly responsive and their linear behavior was proportional to the area of their working electrode. In future the surface of the cell culturing chamber will be cultured with cells and the effect of different toxins and chemicals will be tested on the cell samples while improving the design and quality of the sensors. This system may be helpful in preventing the need for testing the drugs and harmful chemicals on living animals on the planet.

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