A low temperature co-fired ceramic based microfluidic Clark-type oxygen sensor for real-time oxygen sensing

Jin Luo^{a,b,*}, Thomas Dziubla^a and Richard Eitel^{c,*}

^a Department of Chemical and Materials Engineering, University of Kentucky, Lexington,
Kentucky, US, 40506

^b State Key Laboratory of New Ceramics and Fine Processing, School of Materials Science and
Engineering, Tsinghua University, Beijing 100084, P. R. China

^c Department of Chemical Engineering & Materials Science, Stevens Institute of Technology,
Hoboken, New Jersey, US, 07030

Abstract

In this paper, a microfluidic platform for real-time monitoring of dissolved oxygen in a flowing microfluidic environment fabricated using low temperature co-fired ceramic (LTCC) technology is described. The fabricated Clark-type oxygen sensor consisted of three electrodes (working electrode, counter electrode and Ag/AgCl reference electrode), a solid-state proton conductive matrix (Nafion 117 membrane) and polydimethylsiloxane (PDMS) as the oxygen permeable membrane (OPM). The use of a solid-state proton conductive matrix as the electrolyte in the design of the oxygen sensor makes it feasible integrate this device in a typical LTCC fabrication process. Cyclic voltammetry and chronoamperometry measurement were used to characterize electrochemical properties of the developed oxygen sensor. The reduction current was linearly related with the dissolved oxygen concentration ranging from 0-8.1 mg/l under different flow conditions (0.0-1.0 ml/min). The residual currents of the oxygen sensor were less than 3.5% of

* Corresponding author: Tel: +086 13691449659; e-mail address: jluuky2014@sina.com (Jin Luo), reitel@stevens.edu (Richard Eitel)

that measured in oxygen saturated state, and the average response time was 10.9 s. The current device represents an improved Clark-type oxygen sensor with the advantages of easy fabrication, flexible configuration, fast response time, incorporation of microfluidic analyte introduction and real-time detection of dissolved oxygen. The potential applications include material synthesis, cell culture, biological assays incorporating controlled introduction of reagents or analytes and real-time monitoring of dissolved oxygen in a microfluidic environment.

Keywords

Clark-type; Oxygen sensor; LTCC; Proton conductive electrolyte; Electrochemistry

1. Introduction

Oxygen delivery and accurate measurement of the dissolved oxygen concentration are very important in environmental monitoring, biological assays, bioreactors and multiple other applications[1-7]. Oxygen sensing has been frequently used to monitor the cellular metabolic activity and rapidly determine cell viability [2, 4, 8]. Adequate supply of oxygen plays a key role in cell proliferation and differentiation, and abnormal supply of oxygen may lead to a diseased state[9]. Monitoring cellular oxygen consumption rates and cellular respiration provides important information for metabolism, apoptosis, mitochondrial function, toxicological responses to various drugs in the critical biochemical study[1, 9]. In addition, there is an increasing need for the development of low-cost, flexible oxygen sensor with easy and rapid fabrication as the integrated microfluidic devices for biological applications.

Two common techniques for sensing dissolved oxygen in microfluidic applications are luminescent optical sensing and electrochemical sensing. Both demonstrate high sensitivity to oxygen at low concentration[10-13]. Fluorescent oxygen sensing (such as the XF Extracellular

Flux commercialized by Seahorse Biosciences[14]), has seen widespread adoption due to its inherent stability[15, 16]. However, the applications of this technique are limited by the complicated fabrication and expensive lifetime-sensing apparatus[14, 17, 18]. Electrochemical sensors are widely used and available in a number of styles from various manufacturers[17, 19]. The most common embodiment of the electrochemical sensor is the Clark-type configuration, incorporating a cathode, anode, electrolyte solution and a gas permeable membrane. The advantages of Clark type dissolved oxygen sensor are easy fabrication, low cost and fast response compared with luminescent sensing technique. However, those sensors also have limitations and suffer from design specific problems. For example, the consumption of dissolved oxygen at the working electrode may change the local oxygen concentration. In order to address these limitations, mechanical agitation or circulation of the testing sample may be required[2, 17]. In addition, the thin gas permeable membrane, separating the liquid electrolyte from the specimen, is susceptible to mechanical failure. It is also difficult to adapt the functionality of this type of sensor to continuous monitoring, automation and high-throughput measurements[20]. However, several microfabricated sensors are available for microfluidic and high throughput sensing. These include probe style sensors, such as from Strathkelvin Instruments, incorporating microelectrodes to reduce oxygen consumption and sample volume requirements, and single or multiwell plates, up to 90 wells from Bionas GMBH, incorporating printed microelectrodes. In the current work, these concepts are expanded upon for use in a microfluidic flow cell.

This work presents the design and development of a low temperature co-fired ceramic (LTCC) based microfluidic Clark-type oxygen sensor for the real-time assessment of localized dissolved oxygen. LTCC materials were chosen because of their hermeticity and mechanical durability, highly scalable phototyping/manufacturing methods, and the ability to directly integrate both

electronic and microfluidic in a compact 3D package[21-23]. The developed microfluidic oxygen sensor consists of an LTCC substrate with electrodes for detecting electrochemical signals, the solid-state electrolyte, oxygen permeable membrane (OPM), and a microfluidic channel (sealed to a glass slide). In order to facilitate manufacture and increase shelf life, a solid proton conductive electrolyte was chosen instead of liquid electrolyte. The problem of the oxygen consumption was addressed, by the small electrode area employed and continuous flow of the sample through the microchannel (reducing potential for oxygen depletion). The use of a solid-state proton conductive matrix (PCM) membrane also provides mechanical support for the gas permeable membrane eliminating the risk of mechanical membrane failure.

2. Experimental Procedure:

2.1 Operational principle and device layout/design

In the current embodiment the LTCC based Clark type oxygen sensor is composed of an LTCC substrate with three electrodes (including gold working and counter electrodes and an Ag/AgCl reference electrode), the Nafion solid-state electrolyte, a polydimethylsiloxane (PDMS) OPM, a microfluidic PDMS specimen channel, and a glass cover sealed to the channel. The inlet and outlet for connection to the PDMS microfluidic channel were incorporated into the layout of the LTCC substrate layer. Figures 1 and 2 illustrate the conceptual operation and physical design/assembly (respectively) of the developed sensor. The reaction mechanism of Clark type oxygen sensor is based on the electrochemical reduction of the dissolved oxygen which diffuses through gas permeable membrane to the electrolyte at the working electrode. The reduction of dissolved oxygen at the working electrode is a two-process step[24]. The reaction was summarized by:

The reaction at the counter electrode was production of oxygen:

The final dimensions of the sintered LTCC substrate were 3.8×3.6 mm, and thickness 0.7 mm. The surface area of the working, reference and counter electrodes were 0.2 mm², 0.75 mm² and 1.5 mm² respectively.

2.2 Fabrication and Testing

Fabrication of the microfluidic flow chambers and microelectrodes was performed using standard multilayer co-fired ceramic processing methods. For prototyping purposes, commercially available HL2000 green tapes (Heraeus, Thick Film Division, West Conshohocken, PA) were used as substrate materials. Electrical conductors were printed using TC8101 gold paste (Heraeus) for working and counter electrodes, and TC0307 silver paste (Heraeus) for fabrication of reference electrode. The cavities (inlet and outlet) and channels in the green tapes were cut using a CO₂-laser milling system (VLS 3.50, Universal Laser Systems, Scottsdale, AZ). Green tapes were then laminated together using a uniaxial press (3851, Carver, Wabash, IN). The consolidated laminate was sintered following manufacturer's guidelines. Chemical oxidation with aqueous FeCl₃ (Fisher Scientific) was used to produce the AgCl reference electrode.

A polymeric PCM was used as solid electrolyte. The PCM solution consisted of a mixture of one part of 5 wt.% Nafion 117 (Aldrich) solution and one part of 1.25 wt.% polyvinylpyrrolidone (PVP360, Sigma) and 0.04 wt.% addition of 2,6-bis(azidobenzylidene)-4-Methylcyclohexanone (Santa Cruz Biotechnology). Nafion provided the required proton conductivity.

In order to prepare the surface, the LTCC substrate was dipped into a mixture of 3-(trimethoxysilyl) propyl methacrylate (Acros), ethanol and dilute acetic acid (1:10 glacial acetic

acid:water) in a ratio of 1:200:6 for 3 min. The substrate then was rinsed with ethanol to remove any residual reagent, and dried thoroughly at 60 °C for 5 min. The PCM solution was pipetted on the device surface and dried at 70 °C for 1 h. The thickness of the PCM membrane was ~15 µm, estimated by the amount of PCM solution and the surface area coverage.

The PCM membrane was then covered by an OPM. The OPM was prepared from a mixture of PDMS (polydimethylsiloxane, Sylgard 184, Dow Corning) with curing agent in a weight ratio 10:1. Xylene was used to adjust the viscosity in order to obtain uniform PDMS membrane with desired thickness. The xylene-PDMS mixture (1: 3, v/v) was spread on the LTCC substrate, and spun at 2000 rpm for 1 min. The LTCC substrate was spun at 2000 rpm and. The resulting PDMS membrane was cured at 60 °C for 2 h and the thickness was estimated to be ~ 20 µm using optical microscopy.

The channel layer was fabricated from 1 mm thick PDMS. The channel (width = 2.2 mm, length = 23 mm) was cut using a CO₂-laser milling system. A transparent glass was cut into a suitable size and cleaned in isopropanol and dried. Both the glass and PDMS-channel layer were treated with O₂ plasma, and the plasma treated surface of the PDMS was pressed against on the plasma treated surface of the glass. Irreversible cross-linking between the plasma activated PDMS and glass surface resulted in strong adherence of the two materials.

The PCM membrane was first saturated with water. Argon, oxygen, or argon/oxygen plasma have been used to bond the PDMS and glass-covered LTCC together [25-27]. In this work, The PDMS-LTCC substrate and PDMS-glass substrate were manually aligned and mechanically secured with spring clips instead of irreversible bonding using the plasma method. Commercially available dissolved oxygen meter was purchased from Milwaukee (MW600) to determine the dissolved oxygen in the solution. Electrochemical measurements and characterization were

performed using a potentiostat (MPG-2, Bio-logic) with computerized control and data acquisition software (EC-Lab V10.32).

3. Results and discussion:

3.1 Cyclic voltammetry of the sensor

Cyclic voltammetry measurements were used to determine the bias point for oxygen reduction and verify that there is no other competitive reaction at the selected bias point. Cyclic voltammetry measurements for oxygen reduction in oxygen saturated DI water was performed over a potential range from 0 V to -1.0 V with a scanning rate 100 mV/s, as shown in Figure 3. With the initial application of a negative potential (starting from 0 V), a commensurate change in the current was observed, consisting with a kinetically limited reaction. The plateau current started to appear around an applied potential of -0.8 V. The diffusion controlled region for oxygen reduction was observed to be in the range of -0.8 V to -1.0 V, without other competitive reactions. The subsequently chronoamperometric reduction currents measured at an applied potential -0.75 V were used to characterize the oxygen concentration. The applied pulse potential corresponds to a zone controlled mainly by oxygen diffusion and partially by the electron transfer kinetics.

3.2 Oxygen sensing, flow rate effects and medium effects on measured oxygen concentrations

Initial evaluation focused on the dynamic range of the developed sensor for oxygen saturated and oxygen depleted solutions of Dulbecco's phosphate-buffered saline (DPBS, 4 g NaCl, 0.5 g Na_2HPO_4 , 0.1 KCl and 0.1 g KH_2PO_4 in 500 ml DI water), as shown in Figure 4(a). Chronoamperometry (CA) was used to determine the dynamic behavior of the device. The

measurement was obtained by applying a step potential change from -0 V to -0.75 V and measuring the current response for 1 min. The averaged current detected in oxygen depleted DPBS in static condition was -4.9 ± 1.7 nA (mean $\pm$ SD), and no big change was observed for flow rate 0.2, 0.5, 1.0 ml/min. The absolute value of current greatly increased for oxygen saturated DPBS compared with oxygen depleted solution, and higher flow rate results in higher absolute value of current response. The absolute value of current gradually decreased after the application of a potential step for oxygen saturated DPBS when the solution was not flowing, with the averaged currents in CA curves from 55 s to 58 s -306.5 ± 5.3 nA (mean $\pm$ SD, n=6). However, the current value tested under flow condition reached a steady state. The times for the current to reach the plateau in CA curves were 10 s, 11 s and 14 s, and the averaged currents at steady state were -418.9 ± 1.8 nA, -469.3 ± 2.2 nA and -502.7 ± 2.4 nA (mean $\pm$ SD, n=6) for flow rate 0.2, 0.5, 1.0 ml/min respectively.

Oxygen concentrations in the DI water and DPBS with the same dissolved oxygen were tested using developed oxygen sensor. The CA curves of DI water and DPBS demonstrated the similar trend as shown in Figure 4(b), which indicates the positive and negative ions in DPBS showed no effects on the CA results under static and flow conditions.

Nafion membranes have a wide application due to their high ion conductivity[28]. However a trace amount of cations, such as Na^+ , Ca^{2+} , Li^+ and K^+ will contaminate the Nafion membrane. These cationic ions will replace protons in Nafion, because these cationic ions have higher affinity with sulfonic acid group ($-\text{SO}_3^-$) in Nafion than protons, which will further results in considerably different conductivities[29]. Nafion electrolyte will easily be contaminated by the reagent in the sample solution if the Nafion electrolyte was directly contacted with testing sample, which will leads to discontinuity between different solution and unexpected interference

signal[24, 30]. The advantage of the fabricated sensor was that the solid state Nafion membrane was coated with a gas permeable membrane, PDMS, which acted as protective layer for Nafion electrolyte.

3.3 Calibration curve

The oxygen in DI water was depleted through bubbling N₂ gas, and the dissolved oxygen concentration was adjusted through magnetically stirring the DI water. Solutions of different dissolved oxygen concentration were introduced into the sensor at various flow rates to obtain CA curves. Figure 5(a) shows the resulting CA curves under flow rates of 0.5 ml/min as a representative. Higher current values in CA curves corresponded to larger dissolved oxygen concentration. The dissolved oxygen concentrations tested were in the range from 0.0 to 8.1 mg/l. CA curves are used to obtain calibration curves. Figure 5(b) shows the resulting calibration curves under flow rates of 0.0, 0.2, 0.5, 1.0 ml/min. All calibration curves demonstrated a good linear relationship, with correlation coefficients 99.77%, 99.54%, 99.66%, 99.67% for flow rate of 0.0, 0.2, 0.5, 1.0 ml/min respectively, which indicate negligible or no crosstalk between electrodes. The slope coefficient and residual currents of the fitted calibration curves are shown in table I. The absolute value of slope coefficient increased with increasing flow rate. The average residual currents for oxygen depleted solution measured in oxygen depleted solution were 3.4%, 1.0%, 1.0% and 2.0% of the current measured in oxygen saturated solution for flow rate 0.0, 0.2, 0.5, 1.0 ml/min respectively. The residual currents measured in this experiment were comparable to that of the Wu et al.'s works[31], Park's report[17, 32] and Suzuki's studies[33-35].

The reduction currents in DPBS were also measured to verify the calibration curve, and the data were also fitted well into the calibration curve under different flow rates. In this condition, the

developed oxygen sensor can be used to detect the dissolved oxygen in real time regardless of the testing solution and flow rate.

The crosstalk of oxygen sensor is mainly induced by diffusion of the generated oxygen at the counter electrode to the adjacent working electrode and the gradual deterioration of the electrodes due to the interaction with the intermediate products[36, 37]. Microfluidic integration in the LTCC based oxygen sensor is able to immediately replace fresh sample solution at the working electrode and flush away the generated oxygen and the intermediate product at the counter electrode, which greatly reduce the possibility of the crosstalk in this work. In this context, the developed oxygen sensor demonstrate comparable residual current and high linearity of the calibration curves under different flow conditions.

3.4 Response time

The response time of the LTCC based Clark type oxygen sensor was measured through monitoring current change after injection of 0.1 M sodium sulfite (Na_2SO_3). Dissolved oxygen in DPBS can be easily depleted by adding sodium sulfite. A 90% response time was defined as the time for the current to decrease 90% of the value measured at oxygen saturated state when the sensor changed from oxygen saturated state to oxygen depleted state. The Figure 6 shows the current change when 0.1 M sodium sulfite was pipetted into the air-filled channel, which was used in order to effectively remove oxygen around the sensitive area of working electrode. The current measured in air-filled channel was -692.6 ± 2.1 nA (mean $\pm$ SD), which was much higher than that measured when the channel was filled with oxygen saturated DI water or DPBS. This was due to higher solubility of oxygen in Nafion than that of water. The solubility of oxygen in Nafion is 159.4 mg/l, almost twenty times of that in water[38, 39]. When the 0.1 M sodium

sulfite was injected into the channel, the current value decreased to -6.6 ± 1.4 nA (mean $\pm$ SD).

The measured response time was 10.9 ± 0.6 s (mean $\pm$ SD, n=7).

3.5 Comparison with the literatures:

The sensor of the current work is compared to Clark-type oxygen sensors from the literature, summarized in Table II. The LTCC based microfluidic oxygen sensor developed in this work provided comparable response time and residual current compared with the other Clark-type oxygen sensors. Beyond sensor performance, a solid state Nafion electrolyte and PDMS gas permeable membrane were successfully integrated into an LTCC package. The resulting accomplishment enables realization of an integrated microfluidic Clark-type oxygen sensor and capability of real-time monitoring of the dissolved oxygen. The effects of localized consumption of the dissolved oxygen at the working electrode were shown to be insignificant, under both static (no-flow) and flow conditions. At the same time, it offered rapid and inexpensive fabrication and a more flexible platform for detecting dissolved oxygen.

Clark-type oxygen sensors previously reported in the literature were only capable of detecting dissolved oxygen in a static environment. In prior examples, the presence of a flowing test sample led to signal shift or change. The device developed in the current work was capable of oxygen delivery and real-time detection of dissolved oxygen in a flowing medium over a range of flow rates (0.2-1.0 ml/min) with minimal shift (1% change in calculated oxygen concentration).

4 Conclusions

This work describes design, fabrication and characterization of a LTCC based microfluidic Clark-type oxygen sensor. Precise oxygen determination and real-time detection of dissolved oxygen was realized in this integrated microfluidic device. The devices were characterized by

using cyclic voltammetry and CA measurement in both DI water and DPBS, and no effects of the dissolved ions present in DPBS was observed on the results. The calibration curves of the oxygen sensor shows good linearity with correlation coefficient higher than 99.5% for flow rates 0.0, 0.2, 0.5, 1.0 ml/min. The 90% response time of the developed oxygen sensor was 10.9 s. The fast response of the oxygen sensor is beneficial to monitor variation of the oxygen concentration in the biological samples. The usage of the solid-state electrolyte extended the lifetime of the oxygen sensor and realized the microfluidic function in the Clark-type oxygen sensor. The amperometric performance of the developed oxygen sensor is comparable to the sensors described in the literature, with rapid and inexpensive fabrication and a more flexible platform for detecting of dissolved oxygen. The incorporation of an integrated Clark-type oxygen sensor in a microfluidic platform is a unique improvement for potential application in biological assays and bioreactors.

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Tables

Table I. Slope coefficients and residual currents of the linear fitted calibration curves under different flow rates.

Flow rate (ml/min)	0.0	0.2	0.5	1.0
	(n=6)	(n=6)	(n=6)	(n=6)
Slope coefficient (nA/(mg/l)) (mean $\pm$ SE)	-36.25 $\pm$ 0.31	-52.95 $\pm$ 0.26	-58.08 $\pm$ 0.30	-60.42 $\pm$ 0.25
Residual current (nA) (mean $\pm$ SE)	-9.25 $\pm$ 1.22	-10.78 $\pm$ 1.07	-9.56 $\pm$ 1.20	-8.68 $\pm$ 1.10

Table II: Comparison of the properties of LTCC based microfluidic Clark-type oxygen sensor and Clark-type oxygen sensor from literatures. PP: Polypropylene; FEP: Fluorinated ethylene propylene.

Response time (s)	Residual current	Electrolyte	OPM membrane	Type of geometry	Testing Method	Reference
10.9	<3.5%	Nafion	PDMS	Planar	Flowing	This work
6.8	~18%	Liquid	PDMS	Planar	Dipping	2
13.4	~1.0%	Liquid	PDMS	Planar	Dipping	31
4.9	~2%	Liquid	PDMS PP	Planer	Dipping	32

FEP						
9.4	~1.3%	Liquid	PDMS	Planer	Dipping	17
20	0.7%	Liquid	Silicone Rubber	Microprobe	Dipping	35
20-50	-	Liquid	Silicone Rubber	Microprobe	Dipping	5
-	~3%	Nafion	PTFE	Microprobe	Dipping	24
-	40%	Nafion	No Membrane	Microprobe	Dipping	30

Figure Captions

Figure 1. Conceptual presentation of the LTCC based microfluidic Clark-type oxygen sensor and schematic illustration of the reaction mechanism: Reference Electrode (RE), Working Electrode (WE) and Counter Electrode (CE).

Figure 2. (a) Schematic demonstration of a LTCC based Clark-type oxygen sensor. (b) Image of the bonded LTCC based microfluidic Clark-type oxygen sensor.

Figure 3. Cyclic voltammetry of oxygen reduction was measured with DI water filled in microfluidic channel from 0 V to -1.0 V with scanning rate 100 mV/s.

Figure 4. (a) CA of oxygen saturated DPBS under different flow rate, and CA of oxygen depleted DPBS; (b) CA of the oxygen saturated DI water, DPBS under different flow rate.

Figure 5. (a) CA curves of the DI water with different oxygen concentration under flow 0.5 ml/min, and (b) Calibration curves obtained from the results of CA curves with different dissolved oxygen under flow rate 0.0, .02, 0.5, 1.0 ml/min.

Figure 6. Response curve of Clark-type oxygen sensor were tested by injection of the zero - oxygen solution, 0.1 M sodium sulfite. The response time was defined as the time for the current to decrease 90% when the sensor changed from oxygen saturated state to oxygen depleted state.