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Oxidase-Coupled Amperometric Glucose and Lactate Sensors with Integrated Electrochemical Actuation System

Jongwon Park

Chang-Soo Kim
Missouri University of Science and Technology, ckim@mst.edu

Minsu Choi
Missouri University of Science and Technology, choim@mst.edu

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Jongwon Park, *Student Member, IEEE,* Chang-Soo Kim, *Senior Member, IEEE*, and Minsu Choi, *Member, IEEE*

***Abstract*—Unpredictable baseline drift and sensitivity degradation during continuous use are two of the most significant problems of biosensors including the amperometric glucose and lactate sensors. Therefore, the capability of on-demand *in situ* calibration/diagnosis of biochemical sensors is indispensable for reliable long-term monitoring with minimum attendance. Another limitation of oxidase enzyme-based biosensors is the dependence of enzyme activity on the background oxygen concentration in sample solution. In order to address these issues, the electrolytic generation of oxygen and hydrogen bubbles were utilized 1) to overcome the background oxygen dependence of glucose and lactate sensors and 2) to demonstrate the feasibility of *in situ* self-calibration of the proposed glucose and lactate sensors. Experimental data assure that the proposed techniques effectively establish the zero calibration value and significantly improve the measurement sensitivity and dynamic range in both glucose and lactate sensors.**

***Index Terms*—Actuator, calibration, glucose oxidase (GOD), lactate oxidase (LOD), solid-state biosensor, thin-film amperometric sensor, water electrolysis.**

I. Introduction

CONTINUOUS monitoring of biochemical analytes such as glucose and lactate is very valuable for critically ill patients to be used for therapeutic decisions and disease prognosis classification [1]. Lactate is a substrate of great interest in clinical and sport medicine. The blood lactate concentration is generally related with an anaerobic metabolism when the muscle is contracted. The lactate concentration up to 2 mM is considered normal, but many pathologic reasons can increase it [2]. The increased lactate level reflects an imbalance between lactate production, which serves as an indication of inadequate tissue oxygenation. Levels of lactate greater than 4 mM have been shown to be associated with circulatory failure, cardiac arrest, and emergency-department patients. Also, the understanding of the correlation between the level of lactate and aerobic performance is very important to increase athlete's endurance [3]. Glucose is important to many cells as it serves in metabolic energy production. Glucose levels are commonly measured to diagnose diabetes or to monitor adequacy of diabetic control. Its levels up to 100 mg/dl are considered normal. Diabetes is a very common disease, affecting about 2% of the general population, which results from insulin deficiency or insensitivity of the body to the level of insulin present. Glucose sensing is of major significance not only in clinical analysis for the diagnosis and therapy of diabetes [4] but also in biotechnology and in the food industry [5].

In order to achieve the goal of real-time monitoring of lactate and glucose, a reliable sampling and analysis system must be developed to meet the necessary clinical requirements such as size, response time, specificity, sensitivity, reliability, and biocompatibility. In the last two decades, enzyme-based amperometric biosensors have played an increasing role in solving the analytical and clinical problems. However, there are significant problems of most biosensors, including both amperometric glucose sensors and lactate sensors: 1) unpredictable baseline drift and 2) sensitivity degradation during continuous use. Therefore, the capability of on-demand *in situ* calibration and diagnosis of biochemical sensors is desired for reliable long-term monitoring with minimum attendance.

Another major limitation of oxidase enzyme-based biosensors is the dependence of enzyme activity on the background oxygen concentration in sample solutions. For example, lactate oxidase (LOD)-based sensors require oxygen as the cosubstrate to complete lactate oxidation. When the oxygen partial pressure was decreased from 40 to 10 mmHg at 10-mM lactate, nearly a 25% decrease in output was observed during *in vitro* measurement [6]. Several approaches have been made to solve this problem. Electrolytic pulse techniques were proposed to generate oxygen electrolytically near the sensor to enable the glucose sensor operation in an oxygen-deficient solution or to minimize the signal instability caused by the fluctuating ambient oxygen in sample solution [7], [8]. Other approaches involved manipulation of the enzyme membranes, including 1) use of electron mediators to complete the enzyme reaction without oxygen [9], 2) perm-selective diffusion membranes to increase the oxygen/glucose diffusion ratio [10], and 3) oxygen-rich enzyme membranes to supply necessary oxygen from the membrane materials themselves [11]. These improvements have been mainly targeted to minimize the oxygen dependence of sensor signals. However, the problem of baseline drift during long-term monitoring still remains unsolved.

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J. Park and M. Choi are with the Department of Electrical and Computer Engineering, University of Missouri–Rolla, Rolla, MO 65409-0040 USA (e-mail: jp7r9@umr.edu; choim@umr.edu).

C.-S. Kim is with the Department of Electrical and Computer Engineering and the Department of Biological Sciences, University of Missouri–Rolla, Rolla, MO 65409-0040 USA (e-mail: ckim@umr.edu).

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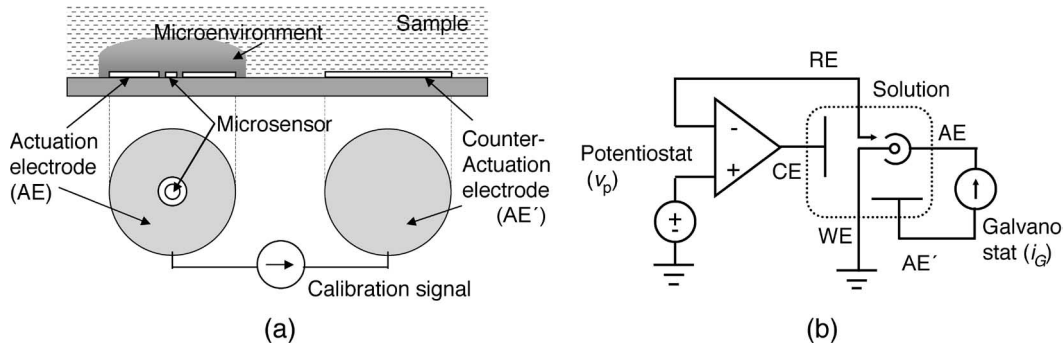

Fig. 1. (a) Concept for a novel oxygen sensor with *in situ* self-diagnosis capability. The microenvironment is generated by an AE that surrounds the microsensor. Oxygen-saturated or oxygen-depleted phases can be established by water electrolysis, depending on the polarity. (b) Potentiostat and a galvanostat are employed for biasing the three-electrode oxygen microsensor and for generating microenvironments, respectively [15].

In this paper, we will demonstrate the following sensor functions to overcome the major shortcomings of the enzyme-based amperometric biosensors mentioned so far: 1) one-point self-calibration (zero-value) capability, 2) extension of linear detection range, and 3) increase in sensitivity in both glucose and lactate sensors. Experimental data assure that the proposed oxygen-controlling microenvironment is effective in both sensors.

In the following section, the previously proposed oxygen-controlling microactuation concept and sensitivity-enhancement technique via oxygen generation are briefly reviewed. Then, the proposed microsensor design with integrated electrochemical actuation system for glucose and lactate monitoring is presented and discussed in Section III. Experimental data from both of the proposed glucose and lactate sensors are shown and discussed in Section IV. Finally, the future work and conclusion are given in Sections V and VI, respectively.

II. Preliminaries and Review

A. O_2-Controlling Microactuator

In order to create a controllable oxygen microenvironment, several electrochemical microactuators, based on water electrolysis, have been reported with the use of micromachining techniques [12]–[14]. Gas pressure was electrochemically generated to be used to change the deflection of a micromechanical diaphragm [12] or to operate an active valve [13]. A micromachined electrochemically driven pump, capable of dosing precise nanoliter amounts of liquid, was introduced as well [14]. Recently, the same water-electrolysis method has been adopted for a novel *in situ* self-diagnosis of the oxygen microsensor [15].

Our group reported a new approach to manipulate the oxygen microenvironment surrounding a dissolved oxygen sensor for *in situ* self-diagnosis capability using electrolytic gas generation via water electrolysis [15]. Dissolved oxygen can be moderately generated or depleted at the actuation electrode (AE) and counter-AE (AE′), as shown in Fig. 1(a).

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

$$4H_2O + 4e^- \rightarrow 4OH^- + 2H_2 \quad (2)$$

where reactions (1) and (2) happen at the anodic AE and at the cathodic AE, respectively.

Accumulation or depletion of dissolved oxygen near the AE, in turn, rapidly establishes a microenvironment of oxygen saturation or depletion. A microsensor, in close proximity to the surrounded AE, can be confined in a controlled local environment. The functionality of the sensor at a high and a low concentration can then be checked in the oxygen-saturated and in the oxygen-depleted phases, respectively. These transient perturbations of the microenvironment equilibrate rapidly with the surrounding medium.

During this first phase of development, an electrolytic AE was integrated in a close proximity to the working electrode of an amperometric dissolved oxygen sensor that has no oxygen permeable membrane. The proposed design exhibited significant artifacts of the local supersaturation of generated oxygen and the local pH fluctuation that affected the catalytic activity of working electrodes. Recently, these two artifacts were circumvented by employing a fiber-optic oxygen microprobe that is immune to pH. The microprobe tip was enclosed by the bubbles generated by separated electrodes within a fluidic structure [16].

B. Performance Improvement of Glucose-Sensitive Ion-Sensitive Field-Effect Transistor (ISFET)

Kim *et al.* reported extended upper detection limit and increased sensitivity of potentiometric glucose biosensors by using integrated electrochemical actuators [17]–[19]. These sensors are based on pH ISFET [pH-ISFET, also known as the chemically sensitive field-effect transistor (CHEMFET)]. Operation of the glucose-sensitive ISFET is shown in Fig. 2. An immobilized GOD membrane on top of the pH-sensitive gate layer serves as the recognition component and is selective only to glucose molecules. Enzymatic reaction causes a pH change inside the GOD membrane that is proportional to the glucose concentration, thereby enabling a potentiometric determination of glucose by pH-ISFET.

The conventional glucose ISFET measures the pH variation caused by the dissociation of gluconic acid, which provides low sensitivity due to the low dissociation constant of gluconic acid. An "amperometric stimulation technique," using the electrochemical actuator, was proposed to overcome this problem.

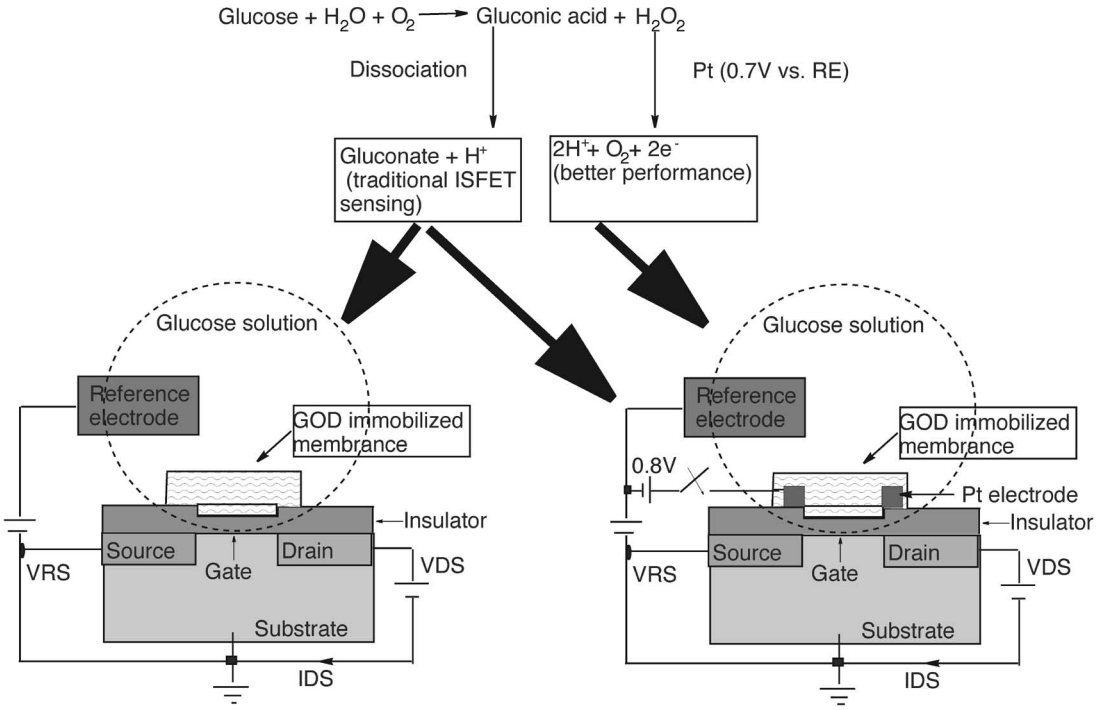

Fig. 2. Operational principle of the glucose-sensitive ISFET. Enzymatic reaction of glucose molecules inside the glucose-oxidase (GOD) membrane causes a pH change proportional to the glucose concentration. The pH-ISFET detects this local pH change to determine the glucose concentration (left). By incorporating a gate-surrounding platinum actuator, the hydrogen peroxide (byproduct) provides two more hydrogen ions per glucose molecule. The generated oxygen contributes to the expedition of the GOD reaction.

Two additional hydrogen ions are produced by the electrolysis of the hydrogen peroxide with the integrated platinum microelectrode, as shown in Fig. 2. With this new mechanism, which can provide two additional hydrogen ions per glucose molecules, the sensitivity and the detection range have been dramatically improved when compared to the conventional sensing mechanism. The generation of oxygen during the decomposition of the hydrogen peroxide also significantly contributed to expedite the enzymatic reaction. These results obtained with the ISFET strongly suggest that both the detection range and sensitivity of amperometric biosensors for glucose and other saccharoids can also be improved with the aid of oxygen-generating electrochemical actuators.

III. Proposed Microsensor With Integrated Electrochemical Actuation System for Glucose and Lactate Monitoring

A. GOD and LOD Enzyme Reactions

The proposed glucose and lactate sensors are based on the amperometric detection of hydrogen peroxide generated by the glucose (or lactate) oxidase-catalyzed oxidation of β-D-glucose (or L-lactate). These enzymes catalyze the following reactions:

$$\beta\text{-D-Glucose} + O_2 \xrightarrow{\text{GOD}} \text{Gluconic acid} + H_2O_2 \quad (\text{GOD: Glucose oxidase}) \tag{3}$$

$$\text{L-Lactate} + O_2 \xrightarrow{\text{LOD}} \text{Pyruvate} + H_2O_2 \quad (\text{LOD: Lactate oxidase}). \tag{4}$$

For each case, the generated hydrogen peroxide is amperometrically detected by the working electrode that has a positive bias (0.85 V) with respect to the reference electrode.

A pair of oxygen and hydrogen bubbles can be reproducibly generated by the water electrolysis with a pair of AEs operating in a constant-current mode. The electrolysis reactions occurring at the anodic and cathodic electrodes are described in (1) and (2), respectively.

The one-point sensor calibration (i.e., zero value) can be performed by manipulating the hydrogen-gas bubble that is generated at the cathodic electrode. The enzyme reaction within the membrane needs oxygen, as shown in (3) and (4). Once the hydrogen-gas bubble is built up on the AE, a carefully driven movement of solution can place the generated hydrogen-gas bubble over the location of the working electrode. When the sensor is surrounded by the hydrogen bubble, this oxygen-free environment prevents the enzyme reaction. This technique results in a glucose (or lactate)-free microenvironment regardless of the actual presence of substrates in sample solutions. On the contrary, the oxygen-gas bubble that is generated from the anodic AE can be used for the measurement-sensitivity enhancement. This artificial constant oxygen environment provides enough oxygen for enzyme reaction such that the enzyme reaction is not limited by the oxygen tension in the sample solution anymore.

B. Materials and Sensor Preparation

The proposed amperometric sensors use glutaraldehyde to crosslink glucose oxidase (GOD) or LOD with bovine serum albumin (BSA). $\beta-$D(+) glucose (EC 207-756-2),

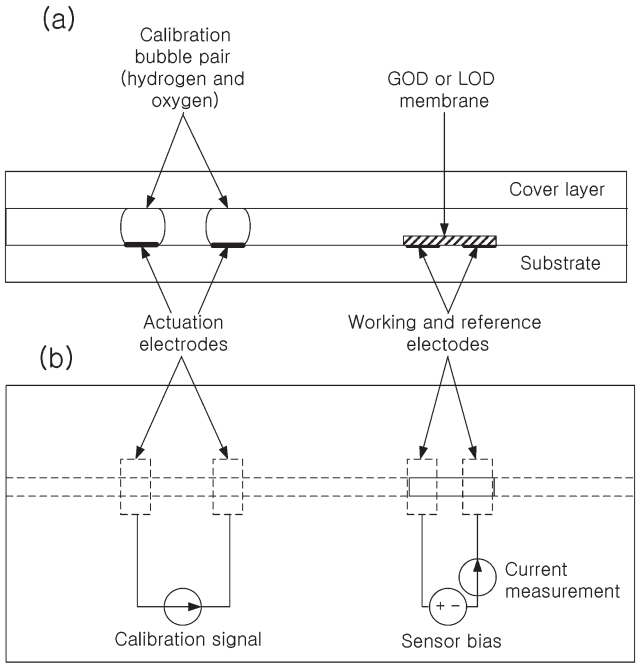

Fig. 3. Proposed fluidic chip for built-in one-point *in situ* calibration of glucose sensor using water electrolysis. Electrochemically generated bubbles provide microenvironment for the one-point calibration and sensitivity-enhancement procedure. (a) Simplified cross section and (b) layout of the system.

L(+) lactate acid (EC 201-196-2), GOD (EC 1.1.3.4, 15500 units/g), BSA (BSA, EC 232-936-2), LOD (EC 232-841-6, 29 units/mg), and glutaraldehyde (EC 203-856-5) were obtained from Sigma (Sigma Chemical Co., St. Louis, MO). All other chemicals were of analytical-reagent grade. Deionized water was used throughout the experiments for the preparation for the samples, buffers, and other solutions.

In general, thicker enzyme layers result in better linearity over a wide concentration range, but the response time is longer. Smaller glutaraldehyde ratio may lead to an inefficient immobilization, while higher ratio may lead to excessive crosslinking and blocking of some active sites of the enzyme [20]. The enzyme solution was prepared by mixing 1.0 mg of BSA in a 10 μl of 10-mM phosphate bubbler solution. To promote adhesion between the enzyme membrane and the electrode surface, a small amount of 1-wt.% 3-aminopropyltriethoxysilane (3-APTES) was applied and cured for 30 min at 80 °C for surface silanization. Next, 10 μl of the enzyme solution was cast on the silanized area with a microsyringe (7000 Series, Hamilton). Then, 10 μl of 5-wt.% GA was applied to initiate the chemical crosslinking reaction of BSA on the electrode surface. For the glucose sensor, the enzymatic enzyme solution included 0.5 mg of GOD and 0.5 mg of BSA in a 10 μl of 10-mM phosphate buffer solution. In a 10 μl of 10 mM phosphate buffer solution, 0.2 mg of LOD and 2.0 mg of BSA were added as a lactate sensor.

Each glucose and lactate sensor chip consists of 1) a cover layer, 2) substrate, 3) sensor electrodes, and 4) AEs. Fig. 3 shows a layout of the assembled system, both cross section and top view. A thick photoresist (MicroChem, SU-8) was used to prepare a template for the molding process of a polydimethylsiloxane (PDMS) cover layer (Corning, Sylgard 184) to include the channel structure. The thick photoresist was patterned on a boro-silicate glass substrate to have a thickness of 100 μm. The PDMS was cast onto the glass substrate and cured for 24 h at room temperature in a vacuum desiccator. The thickness of the PDMS cover layer was about 0.75 cm. The substrate was a silicon wafer with a silicon-nitride-layer coating. A platinum/titanium thin film (100 nm/20 nm) was deposited by e-beam evaporation and patterned by lift-off technique to define the electrodes. The PDMS cover layer was attached to the substrate by simply pressing against the substrate to seal the interface between the PDMS and the silicon nitride layer. The large patterns of the channel and the actuator electrodes allowed manual alignment of the cover layer with the substrate. A photograph of fully assembled microsensor is shown in Fig. 4.

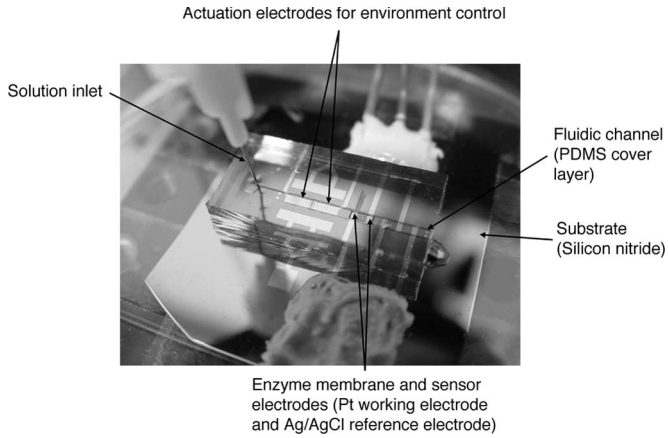

Fig. 4. Photograph of the fully assembled fluidic chip.

C. Sensor Operation

Each time of operation, the proposed microsensor measures the output responses under three different microenvironments: air saturated, O_2 saturated (in an O_2 bubble), and O_2-depleted (in a H_2-bubble). Various glucose control solutions (0, 50, 100, 200, 300 mg/dl) and lactate control solutions (0, 18, 36, 54, 72, 90 mg/dl) are used to check the functionality of the sensor. They were prepared by dissolving each substrate into a phosphate buffer solution. The microsensor operation consists of the following steps.

1) The microfluidic channel is initially filled with air-saturated glucose or lactate solution under measurement from the glucose-solution inlet.
2) Embedded electrodes generate both H_2 and O_2 bubbles in the microfluidic channel by water electrolysis [Fig. 5(a)].
3) The first output response is initially measured in the air-saturated microenvironment in the channel.
4) Each solution in the syringe is pressurized so that the O_2 bubble is placed over the sensor. Then, the second output response is measured in the O_2-saturated microenvironment in the channel [Fig. 5(b)].
5) Each solution in the syringe is pressurized once again so that the H_2 bubble is placed over the sensor. Then, the third output response is measured in the O_2-depleted microenvironment in the channel [Fig. 5(c)].

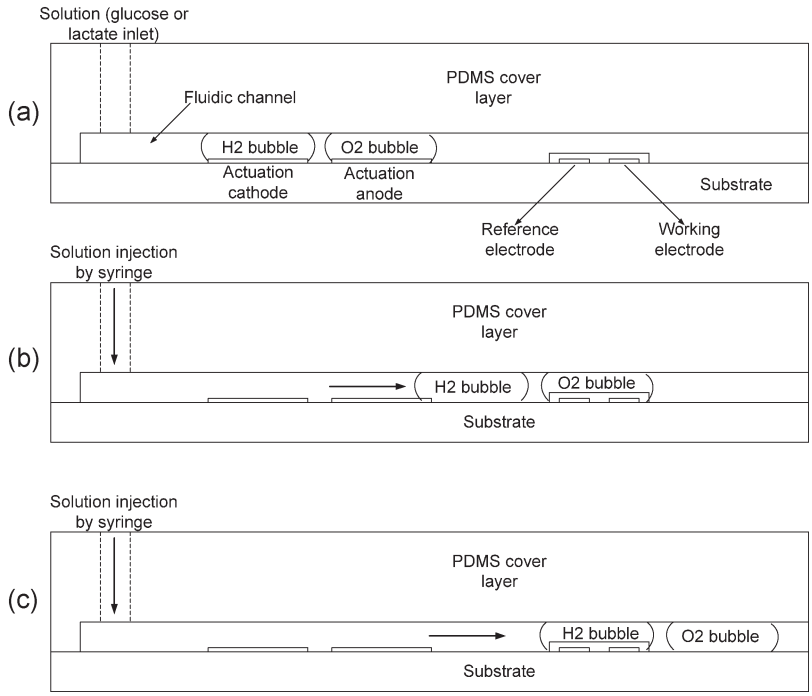

Fig. 5. (a) Generation of H_2 and O_2 bubbles using water electrolysis. (b) Sensor-output measurement under O_2-saturated microenvironment for enhanced sensitivity. (c) Sensor-output measurement under O_2-depleted microenvironment for *in situ* one-point self-calibration (zero value).

In Fig. 6, the following are shown: 1) three different controlled microenvironment phases (i.e., air saturated, O_2 bubble, and H_2 bubble) provided by AEs, 2) chronoamperometric pulsatile operation of the proposed sensors, and 3) their time responses for glucose Fig. 6(a) and lactate Fig. 6(b) sensors. A potential pulse was applied to the working electrode (+0.85 V versus reference electrode) to detect hydrogen peroxide generated from the glucose or lactate reaction. To avoid biased signal (i.e., constant-current signal for bubble generation) induced from AE, a chronoamperometric [i.e., constant voltage (0.85 V)] pulse signal was applied to measure each substrate after the generation and movement of the gas bubble within the fluidic channel. The time responses shown in Fig. 6(a) and (b) were obtained from 200-mg/dl glucose and 72-mg/dl lactate sample solution, respectively.

IV. Glucose and Lactate Measurements

An electrochemical instrument (Gamry Instruments, FAS1) was used to provide the chronoamperometric operation for the sensor (i.e. constant-voltage mode) and the galvanostatic operation for the water-electrolysis actuation (i.e., constant-current mode). The output current of the sensor was measured with respect to the concentration of the glucose and the lactate. During the experiment, a microscope with a charge-coupled-device (CCD) camera was used to check the images of calibrant bubble generation and motion in the channel.

Figs. 7 and 8 show the calibration/measurement curves obtained with the prepared glucose and lactate sensors, respectively. The lower curves in these plots were obtained when the sensors were enclosed in a hydrogen bubble (i.e., 0% oxygen environment). Since the oxygen is not available within the hydrogen bubble, the enzyme (GOD or LOD) reaction cannot be completed, which means that the sensor output is zero regardless of the actual glucose or lactate in the sample solution. Therefore, the proposed *in situ* one-point calibration (i.e., zero value) of each sensor is possible in a hydrogen bubble. Also, as shown in the upper curves, the dynamic range and the sensitivity were improved when the sensor was enclosed in an oxygen bubble (i.e., 100% oxygen environment) compared to the middle curves obtained in normal air-saturated sample solutions (i.e., 21% oxygen environment). In an oxygen bubble, the enzyme reaction is not limited by the oxygen supply, and the reaction is independent from the background oxygen content in the sample solution.

In Fig. 7, linear trendlines (LTLs) of the glucose sensor-output curves under O_2-saturated and air-saturated microenvironments are also shown (e.g., LTL of enhanced output and LTL of normal output, respectively). The LTL of the enhanced-output curve is $y = 15.571x + 580.81$ and $R^2 = 0.9082$, while the LTL of the normal-output curve is $y = 3.0468x + 160.57$ and $R^2 = 0.8946$. Notably, the measurement sensitivity of the sensor is significantly improved since the slope of the LTL of the normal curve is enhanced from 3.0468 to 15.571. The enhanced-output curve obtained from the O_2-saturated microenvironment makes more accurate glucose-level measurement possible. For even more confidence in sensor-output reading, a multidegree polynomial trendline (PTL) can be modeled. For example, a third-degree PTL, $y = 0.0003x^3 - 0.1827x^2 + 43.887x - 117.69$, is shown as well. It has a significantly improved R^2 value (e.g. the goodness-of-fit measure) of 0.9779.

Another similar analysis was performed for the lactate sensor, and results are shown in Fig. 8. The LTL of the enhanced-output curve is $y = 93.876x + 275.6$ and $R^2 = 0.7888$. It has a significant improvement in the measurement sensitivity over the LTL of the normal curve with

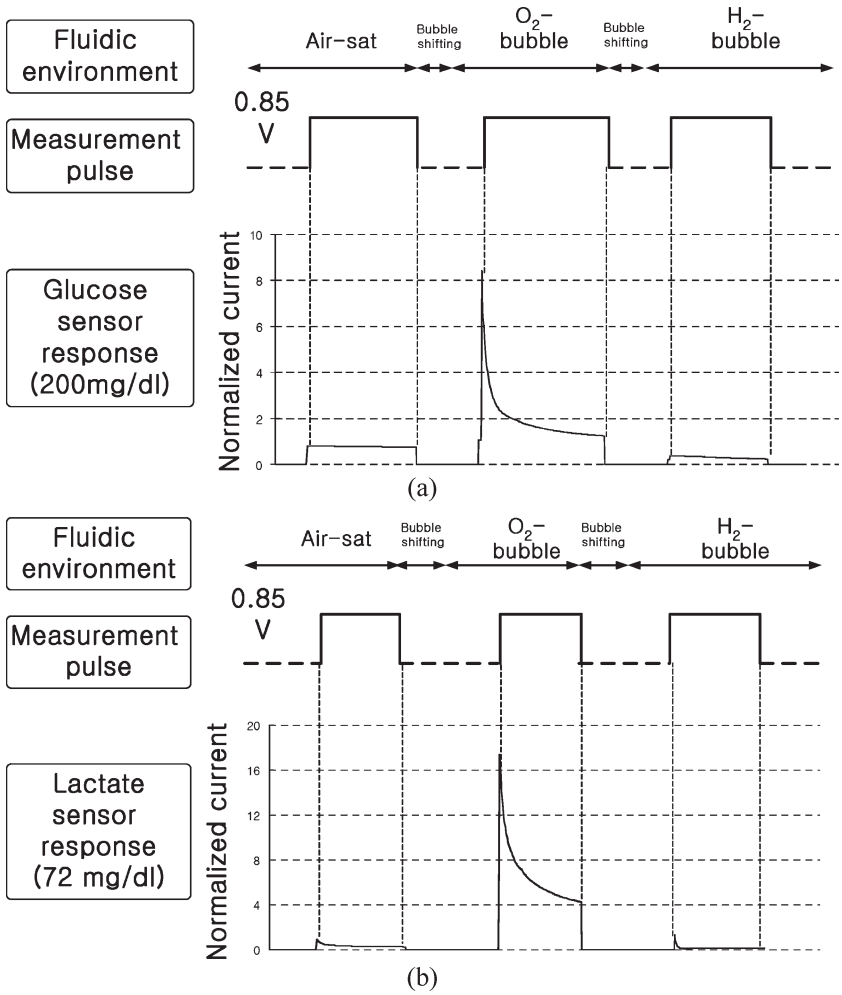

Fig. 6. Three different controlled microenvironment phases provided by AEs, chronoamperometric pulsatile operation of sensors, and their time responses of (a) glucose and (b) lactate sensors. A potential pulse was applied to the working electrode (+0.85 V versus reference electrode) to detect the hydrogen peroxide generated from the glucose or lactate reaction.

$y = 6.6196x + 19.02$ and $R^2 = 0.7853$. Its third-degree PTL $y = 3.2961x^3 - 63.48x^2 + 414.89x + 9.3087$ is also shown. It has a significantly improved R^2 value of 0.997.

There are various enzyme-immobilization methods available using different chemical agents with different compositional ratios [20]. Mulchandani *et al.* [21] have recently reported that LOD is not amenable to glutaraldehyde crosslinking. These observations suggested that LOD immobilization with glutaraldehyde crosslinking required a proper control of the enzyme-mixture composition. This is considered to be the primary reason of the low sensitivity of the lactate sensor in the air-saturated microenvironment. Further standardization of the enzyme-membrane layer will be necessary to manipulate the appropriate sensitivity and linear range for practical applications.

V. Future Work

In both glucose and lactate cases, the proposed microsensors successfully demonstrate the desired capability of one-point self-calibration and significant enhancement in measurement sensitivity. It is also anticipated that the same technique can be extended for the other saccharoids. Simultaneous measurement of glucose and lactate is of great importance for patient monitoring, as well as for quality control in food production and process control in biotechnological plants. This multianalytical sensing should be fouling free and interference free, as done in the dual electrode amperometric biosensor integrated with a macrodialysis sampling system proposed by Palmisano *et al.* [22]. Otherwise, the multiple sensing elements in a close proximity may interfere each other and may result in inaccurate measurements. In our future work, we plan to integrate multiple-saccharoid sensing elements and the proposed oxygen controlling actuator in a single chip for multimodal sensing with minimum attention and enhanced-measurement accuracy. Although the results from both of the proposed glucose and lactate sensors are encouraging for the future work, further investigations are needed, especially on 1) relatively nonlinear responses and 2) possible interference and crosstalk among different sensing elements. In our future work, we will extensively address these issues.

VI. Conclusion

This paper has presented novel GOD- and LOD-coupled amperometric microsensors with integrated electrochemical

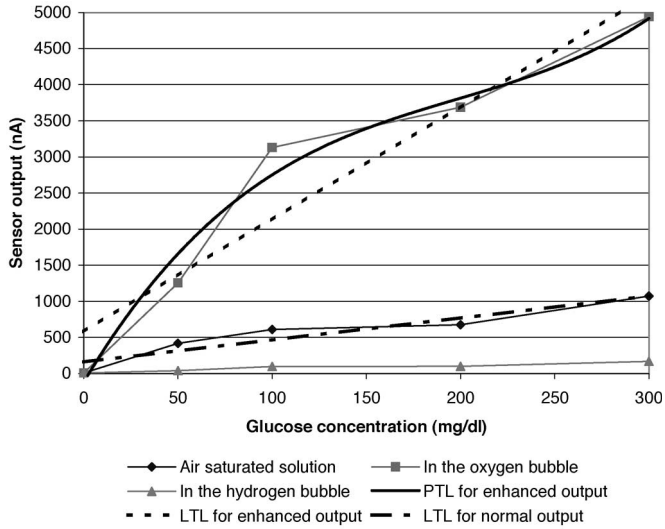

Fig. 7. Glucose sensor calibration/measurement curves. In the hydrogen bubble (i.e., 0% oxygen), the one-point (zero value) calibration is feasible mimicking glucose-free solution regardless of the actual glucose concentrations in the sample solution. In the oxygen bubble (i.e., 100% oxygen), stable responses can be obtained due to the constant background oxygen microenvironment. A third-degree PTL for the O_2-enhanced curve is also shown.

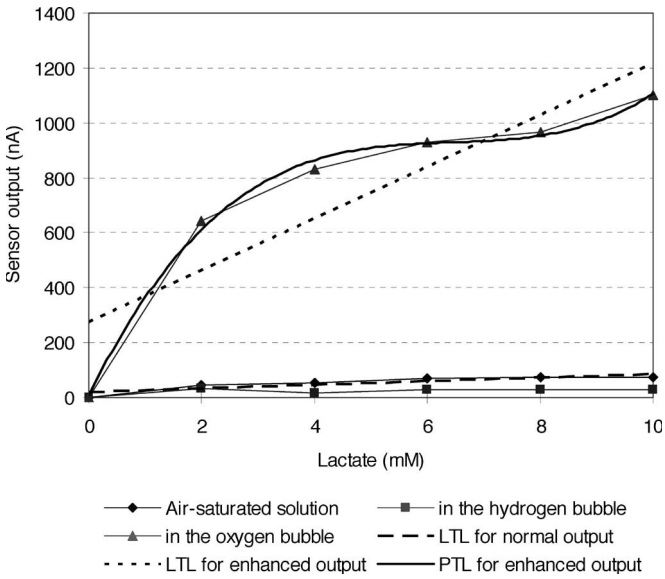

Fig. 8. Lactate sensor calibration/measurement curves. During the hydrogen-bubble phase, the one-point (zero value) calibration is done, since the oxygen-depleted microenvironment provided by the generated hydrogen bubble is mimicking the lactate-free solution, regardless of the actual lactate concentration in the sample solution. The higher sensitivity and wider dynamic range are obtained due to the constant background oxygen environment during the oxygen-bubble phase. LTLs for normal and enhanced curves and a third-degree PTL for the enhanced curve are shown as well.

actuation system. Each of the proposed sensor systems has three embedded components: 1) fluidic channel for glucose or lactate movement, 2) O_2-depleting/saturating built-in electrochemical actuator, and 3) solid-state oxidase-coupled amperometric sensing element. For each sensor, three different output responses can be obtained: air saturated, O_2 saturated, and O_2 depleted. The O_2-depleted output response can be used for *in situ* one-point self-calibration and diagnosis. Also, the O_2-saturated output response can be used to achieve significantly enhanced measurement sensitivity over the normal air-saturated output response.

Two sets of laboratory experiments were conducted on the fabricated prototype sensor systems, and data were collected for different glucose- and lactate-concentration levels. The collection of data verifies that each of the proposed glucose and lactate sensor systems successfully establishes the zero calibration value using the O_2-depleted microenvironment and significantly improves its measurement sensitivity and confidence using the O_2-saturated microenvironment. The proposed sensor systems can be used for continuous glucose- and lactate-level monitoring purposes with minimum attendance.

In our future work, we will integrate two proposed sensing elements (i.e., glucose and lactate) in a single chip for simultaneous and continuous measurements of glucose and lactate, while ensuring acceptable linearity in sensor responses and maintaining interference and crosstalk-free operation.

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Jongwon Park (S'04) received the B.S. degree in aerospace and mechanical engineering from Hankuk Aviation University, Goyang, South Korea, in 1996 and the M.S. degree in electrical engineering from University of Missouri–Rolla in 2005, where he is currently working toward the Ph.D. degree.

He served as an Avionics maintenance officer at the Republic of Korea Air Force. After being discharged, he joined Korea Aerospace Ind. Ltd., South Korea, where he worked on the electromagnetic interference (EMI)/electromagnetic compatibility (EMC) for the T/A-50 aircraft development program. He is currently with the University of Missouri–Rolla.

Chang-Soo Kim (SM'01) received the B.S., M.S., and Ph.D. degrees in electronic and electrical engineering from Kyungpook National University, Daegu, South Korea, in 1989, 1991, and 1997, respectively. During his graduate study, he worked on integrated-circuit technologies and their applications to microsensors, especially for ISFET.

As a Research Associate at the Sensor Technology Research Center, South Korea, he was involved in various research projects and commercial developments of microelectrochemical sensors and systems for the monitoring of gases, electrolytes, and biomolecules. At the Biomedical Microsensors Laboratory at North Carolina State University, Raleigh, and the Experimental Cardiology group, University of North Carolina at Chapel Hill, he conducted postdoctoral research on intelligent biochemical sensors, implantable device platforms, and cardiac biopotential recording with micromachined probes. He joined the University of Missouri–Rolla in 2002 as an Assistant Professor with a joint appointment at the Departments of Electrical and Computer Engineering and Biological Sciences. His current research interests include autonomous microsystem technologies and novel applications of microsystems to plant sciences, metabolic engineering, and space microgravity environment.

Dr. Kim is a member of Biomedical Engineering Society (BMES), Eta Kappa Nu, American Society for Engineering Education (ASEE), and Sigma Xi.

Minsu Choi (M'02) received the B.S., M.S., and Ph.D. degrees in computer science from Oklahoma State University, Stillwater, in 1995, 1998, and 2002, respectively.

He is currently with the Department of Electrical and Computer Engineering, University of Missouri–Rolla, as an Assistant Professor. His research mainly focuses on computer architecture and very large scale integration (VLSI), embedded systems, fault tolerance, testing, quality assurance, reliability modeling and analysis, configurable computing, parallel and distributed systems, instrumentation and measurement, and computational nanotechnology.

Dr. Choi is a member of Sigma Xi and of the Golden Key National Honor Society.