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Novel CeO_2–CuO-decorated enzymatic lactate biosensors operating in low oxygen environments

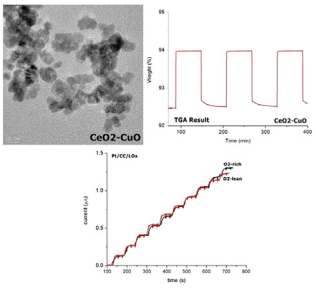
Aytekin Uzunoglu [a, *], Lia A. Stanciu [a, b]

[a] School of Materials Engineering, Purdue University, 701 West Stadium Avenue, West Lafayette, IN 47907, USA
[b] Weldon School of Biomedical Engineering, Purdue University, 206 South Martin Jischke Drive, West Lafayette, IN 47907, USA

HIGHLIGHTS

- Here, we described the use of CeO_2–CuO nanoparticles to address the oxygen dependence challenge of first generation amperometric lactate biosensors.
- The use of CeO_2–CuO mixed metal oxide acted as an oxygen buffer on the sensing platform in oxygen-depleted buffer solution.
- The false results associated with the depletion of oxygen tension in PBS solution were eliminated.

GRAPHICAL ABSTRACT



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ABSTRACT

The detection of the lactate level in blood plays a key role in diagnosis of some pathological conditions including cardiogenic or endotoxic shocks, respiratory failure, liver disease, systemic disorders, renal failure, and tissue hypoxia. Here, we described for the first time the use of a novel mixed metal oxide solution system to address the oxygen dependence challenge of first generation amperometric lactate biosensors. The biosensors were constructed using ceria-copper oxide (CeO_2–CuO) mixed metal oxide nanoparticles for lactate oxidase immobilization and as electrode material. The oxygen storage capacity (OSC, 492 μmol-O_2/g) of these metal oxides has the potential to reduce the oxygen dependency, and thus eliminate false results originated from the fluctuations in the oxygen concentration. In an effort to compare the performance of our novel sensor design, ceria nanoparticle decorated lactate sensors were also constructed. The enzymatic activity of the sensors were tested in oxygen-rich and oxygen-lean solutions. Our results showed that the OSC of the electrode material has a big influence on the activity of the biosensors in oxygen-lean environments. While the CeO_2 containing biosensor showed an almost 21% decrease in the sensitivity in a O_2-depleted solution, the CeO_2–CuO containing electrode, with a higher OSC value, experienced no drop in sensitivity when moving from oxygen-rich to oxygen-lean conditions. The CeO_2–CuO decorated sensor showed a high sensitivity (89.3 ± 4 μA mM^{-1} cm^{-2}), a wide linear range up to 0.6 mM, and a low limit of detection of 3.3 μM. The analytical response of the CeO_2–CuO decorated sensors was studied by detecting lactate in human serum with good selectivity and reliability. The results revealed that CeO_2–CuO containing sensors are promising candidates for continuous lactate detection.

* Corresponding author.
E-mail addresses: auzunogl@purdue.edu, aytekingyte@gmail.com (A. Uzunoglu).

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1. Introduction

An increased lactate level in blood is used to diagnose a higher risk of morbidity both in emergency and surgical operations [1,2]. Moreover, it is known that an increased lactate level is associated with some pathological conditions such as cardiogenic or endotoxic shocks, respiratory failure, liver disease, systemic disorders, renal failure, and tissue hypoxia [3–6]. The first protocol to measure the lactate concentration in blood was introduced in 1886, and relied on the collection of a large quantity of blood sample (100–200 ml) and labour intensive steps [1]. In addition to these challenges, it required several days to complete the measurement [1]. Since then, intensive effort has been devoted to construct small sized devices with shorter turnaround time to measure the blood lactate level using minimal amount of sample. The technological advances in the last couple of decades enabled scientists to invent new methods to detect the lactate concentration less than 2 min [7]. Although the concentration of lactate can be measured using various methods including liquid and gas chromatography, optical analysis, chemical oxidation, and enzymatic oxidation, these methods are complex, require pretreatment and expertise [6,8]. Therefore, construction of inexpensive and simple lactate biosensors with high sensitivity and selectivity, a low limit of detection, and a fast response time, which requires no expertise has gained great attention in the last couple of decades.

The use of amperometric biosensors is an alternative method to conventional approaches for the detection of lactate level in blood [3,9]. Since amperometric biosensors have several advantages over the conventional methods, they have been studied extensively. The detection of lactate using an amperometric enzyme biosensor requires the application of a potential between working and reference electrodes [3]. The main components of the working electrode are enzyme and catalyst layers, which take part in oxidations of lactate and hydrogen peroxide, respectively. To date, lactate oxidase is the most commonly used enzyme as the biological component in enzymatic lactate biosensors [6]. Lactate oxidase catalyses the oxidation of lactate as described Eq. (1) [3].

$$\mathrm{L-Lactate} + \mathrm{O_2} \xrightarrow{LOx} \mathrm{pyruvate} + \mathrm{H_2O_2} \quad (1)$$

The produced H_2O_2 is then oxidized on the catalyst surface according to Eq. (2) at a certain potential [3].

$$\mathrm{H_2O_2} \rightarrow \mathrm{O_2} + 2\mathrm{H^+} + 2\mathrm{e^-} \quad (2)$$

Since the amount of lactate is proportional to H_2O_2 generated by lactate oxidation, its concentration is used to determine the lactate concentration by measuring the current generated from the oxidation of hydrogen peroxide [3,10]. As indicated Eq. (1), oxygen is utilized as the physiological electron acceptor for lactate oxidation reaction. Therefore, the detection of lactate level using these biosensors is highly dependent on the oxygen concentration. On the other hand, oxygen concentration in blood is subject to fluctuations resulting in errors in measured lactate concentrations [11]. Moreover, oxygen deficiency in the blood sample deteriorates the detection range of the sensors [12]. The inadequate oxygen level cannot sustain the oxidation of lactate; thus, fluctuations in the oxygen concentration hinder accurate detection of lactate level. To eliminate the oxygen dependency problems in electrochemical sensors, some approaches have been proposed including the use of two dimensional cylindrical electrodes [13], mass transport limiting films [13,14], and tailoring the electrode surface with oxygen-rich materials [15]. In addition to the modification of electrode using one of the approaches listed above, some authors substituted lactate oxidase with lactate dehydrogenase (LDH) which does not require oxygen as an electron acceptor [3]. On the other hand, the replacement of lactate oxidase with lactate dehydrogenase enzyme requires the immobilization of coenzymes (NADH or NADPH), which enables the electron transfer between enzyme and electrode surface [3]. Since the immobilization of coenzymes makes the sensor construction more complicated in terms of enzyme immobilization and regeneration, publications that used lactate oxidase outnumbered those that used lactate dehydrogenase [3]. In addition, it was reported that lactate dehydrogenase based biosensors showed lower sensitivity compared to the biosensors with lactate oxidase [3,6]. With the introduction of the second generation biosensors, oxygen was replaced with a synthetic electron acceptor known as artificial mediator to make the electrodes oxygen independent [12]. In the case of an artificial mediator, although higher electron transfer rates and lower working potentials can be achieved, the presence of the mediator in the enzyme layer may impair the enzyme stability by creating a toxic environment [3]. Thus, choosing the right mediator, which is not detrimental to enzymatic activity is a key step in the construction of second-generation biosensors. Moreover, the immobilization and stability of the artificial mediator are also challenges to be dealt with [11,16]. In summary, most of the methods used to eliminate the oxygen deficiency related errors in the sensor response pose new problems including sensitivity, selectivity, and stability, require more complex preparation methods and hinder the miniaturization of sensors [17]. Thus, new approaches are on demand to address the oxygen dependence challenge of first generation enzymatic sensors.

In recent years, the use of metal oxide nanoparticles has attracted great interest in several applications such as catalysis, sensors, electronic materials, and environmental materials due to their unique physical, catalytic, and chemical properties [18,19]. Since the properties of the metal oxide nanoparticles are highly dependent upon their size and morphology, they offer vast range of properties for various applications [20]. Their biocompatible nature, enhanced electron-transfer kinetics and strong adsorption capability make them promising materials for the immobilization of biomolecules in biosensing applications [21,22]. Among various metal oxides, ceria has been used in many applications including catalysis, solid oxide fuel cells [23], sensors [24], oxygen permeable membranes [25], glass polishing [26], ultraviolet adsorption [27], and biotechnology [19,28]. Its high ionic conductivity, biocompability, high surface area, high isoelectric point, and catalytic properties generated great deal of interest for biosensor applications [22,29]. Apart from these properties, the unique redox properties of ceria, also known as oxygen storage capacity (OSC), make it an important technological material for many applications [30]. Since cerium (Ce) can exist in two oxidation states, Ce^{4+} and Ce^{3+}, it forms two stable oxide species which are CeO_2 and Ce_2O_3 [31]. By a reversible shift between +4 and +3 oxidation states, ceria releases and uptakes oxygen under oxygen lean and oxygen rich environments, respectively. The reversible redox property of CeO_2 has been exploited extensively as an oxygen buffer material for automotive applications in particular three-way catalysis [30,31]. Much effort has been directed toward preparing new ceria-based nanomaterials with enhanced OSC. It is known that the OSC of ceria is strongly dependent on the specific surface area and oxygen defect concentration in the structure [31,32]. Our previous work showed that the modification of ceria with other metal oxides such as CuO, TiO_2, and ZrO_2 is an effective way to significantly enhance the OSC of ceria [31]. The introduction of ZrO_2 into the CeO_2 lattices, for example, gave rise to the number of oxygen defects in the ceria structure, which in turn enhanced the OSC.

Recently, our group showed that the oxygen deficiency formed in the enzyme layer of amperometric lactate biosensors can be