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Development toward a novel integrated tear lactate sensor using Schirmer test strip and engineered lactate oxidase

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ABSTRACT

L-Lactate is an important biomarker for clinical diagnostics and fitness monitoring that shows oxygen deficiency or elevated salt concentrations due to pathophysiological conditions or intensive exercise. To avoid current painful and inconvenient blood testing techniques of measuring lactate levels, tears represent a non-invasive and potential sampling medium. However, lactate oxidase (LOx) is susceptible to the fluctuation and deficiency of oxygen, leading to inaccurate lactate measurements in tears. By utilizing a recently published, protein engineered LOx that eliminates the oxygen interference, a novel tear lactate (TL) sensor was assembled and tested. Screen-printed sensors were prepared with a redox solution containing the engineered LOx, and a novel tear sampling component made of Schirmer's test strip was attached to absorb the simulated tear fluid samples. The dynamic range of the TL sensor was found to be 0.39–16.60 mM in simulated tear fluid, satisfying the clinically relevant range of TL. In addition, the proposed TL sensor was found to be insensitive to ascorbic acid, acetaminophen, and uric acid, which are common interfering compounds in tears, and showed no sign of degradation after 8 weeks of shelf life study. The proposed sensor exhibited potential usefulness in providing an alternative noninvasive method of measuring lactate and in calibrating the continuous lactate contact lens.

1. Introduction

L-Lactate is an important biomarker often measured in clinical diagnostics [1] and in monitoring the fitness of athletes [2]. Lactate levels in the body indicate oxygen deficiency or elevated salt concentrations and can be altered due to pathophysiological conditions or intensive exercise [3]. Elevated blood L-Lactate concentration can reflect lactic acidosis caused by various factors such as toxins, shock, anemia, sepsis, and organ failure [4]. Healthy individuals can also suffer from elevated L-Lactate levels due to physical strain after prolonged periods of extensive anaerobic activity.

Although there are commercially available lactate meters and test strips [5], the pain and use of needles can be undesirable and inconvenient. Compared to blood, tear fluid is more accessible and less complicated in composition. Tear lactate (TL) level ranges from 1 to 5 mM [6]. This is significantly higher than blood lactate level because tear fluid is utilized as a means for the removal of L-Lactate from body metabolism via diffusion across the stroma and endothelium into the tear film [7]. TL is also not interfered by haematocrit as it is in blood, suggesting that tear fluid may be an ideal alternative for lactate monitoring.

For TL monitoring, there has been a revolutionary development of continuous lactate contact lens [8]. However, similar to how current continuous glucose monitoring systems (CGMS) require the use of blood glucose test strips for calibration, a reliable TL test strip should accompany the continuous lactate contact lens. The detection of lactate typically employs lactate oxidase (LOx) [9]. LOx uses oxygen as a primary electron acceptor for the lactate oxidation reaction [10]. Oxygen deficiency and fluctuation can affect the oxidation of lactate and yield errors in measured lactate concentrations [11]. This phenomenon can be problematic in the ocular environment. Since long term contact lens wearers suffer from various ocular diseases due to significant reduction

Abbreviations: CGMS, continuous glucose monitoring system; CE, counter electrode; CV, cyclic voltammetry; FDA, Food and Drug Administration; LOx, lactate oxidase; RE, reference electrode; SPE, screen printed electrode; Ag/AgCl, silver/silver chloride; TL, tear lactate; WE, working electrode

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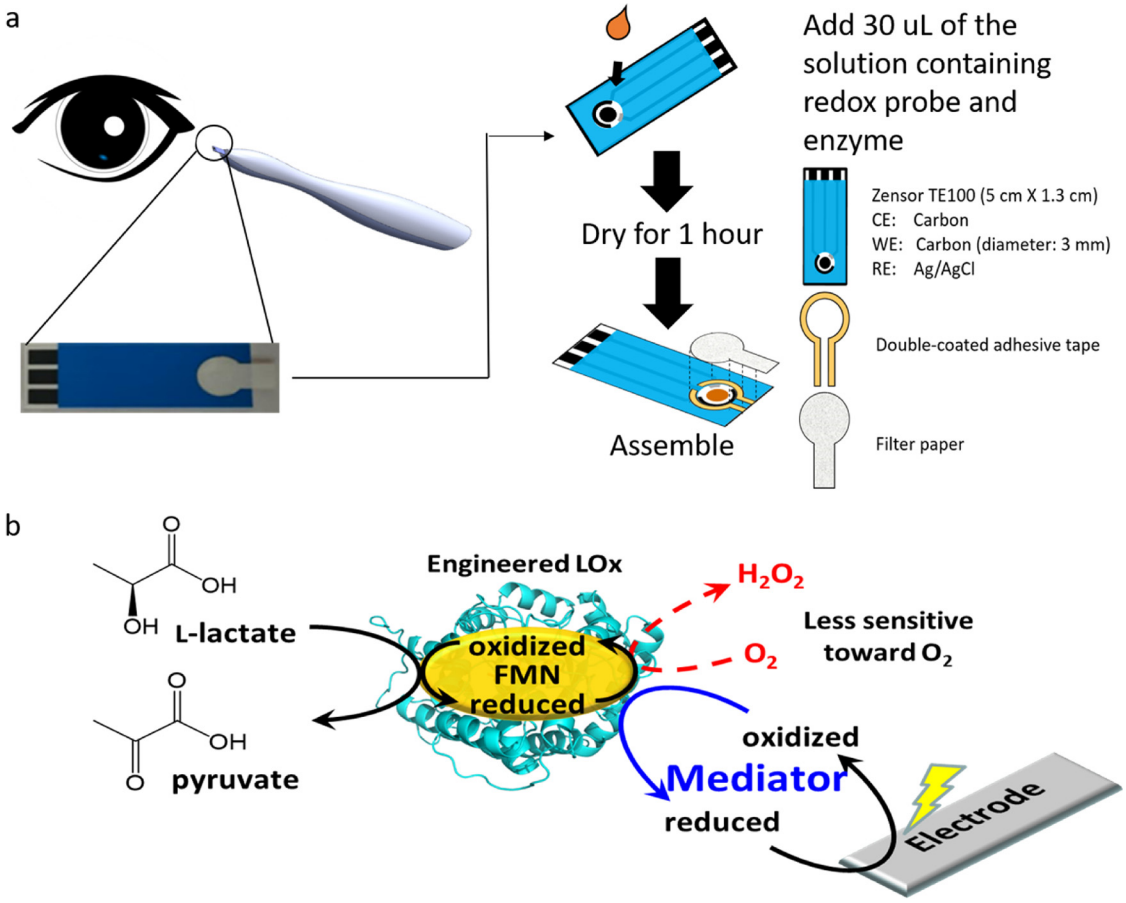

Fig. 1. a) Tear lactate sensor schematic. The tear lactate test strip can be inserted into a pen-like meter designed to ergonomically collect tear fluid by touching the conjunctiva with filter paper. The integrated test strip fabrication process is shown on the right. The Zensor consists of a carbon working electrode (WE), carbon counter electrode (CE), and silver/silver chloride (Ag/AgCl) reference electrode (RE). b) Enzymatic schematic of the engineered lactate oxidase. LOx oxidizes L-lactate to pyruvate through the reduction of its co-factor, flavin mononucleotide (FMN). The engineered LOx, which is much less sensitive toward oxygen, utilizes virtually only artificial electron acceptors to re-oxidize FMN. Reduced artificial electron acceptors can transfer the electrons between the LOx and the electrode.

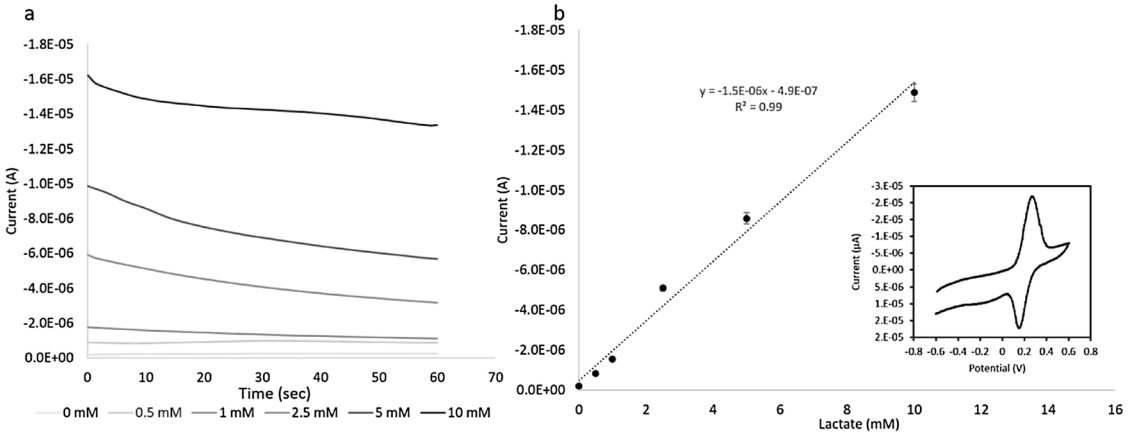

Fig. 2. a) the amperometric-time response of each lactate concentartion in simulated tears. b) the calibration curve of the integrated tear lactate sensor and inset the CV response of the integrated sensor. Concentrations tested were 0, 0.5, 1, 2.5, 5, 10 mM of L-lactate, with 10 replications each. The error bars represent one standard error.

in oxygen uptake, they may be subjected to various degrees of oxygen deficiency or fluctuation in tears [12]. Although current technology has manufactured gas permeable contact lens to ensure sufficient oxygen exchange in tears, risks for oxygen fluctuation or deficiency still persists due to user misuse, including inadequate cleaning, infrequent changing of contact lens, and other lifestyle choices. Therefore, it is important to build a TL sensor that is robust against oxygen fluctuations. An accurate TL disposable test strip can be used to calibrate the continuous lactate contact lens and provide an alternative method for those who prefer not to use contact lens or needles in measuring lactate levels.

Oxygen interference from oxidase enzymes can be removed by employing site-directed mutagenesis to reduce oxidase activity and increase dye-mediated dehydrogenase activity using an artificial electron acceptor [13–15]. Recently, using site direct mutagenesis, Hiraka