Continuous assessment of sweat lactic acid secretion using microfluidic sweat lactic acid monitoring system

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Abstract. A microfluidic sweat lactic acid (LA) monitoring system for non-invasive and continuous assessment of sweat LA was constructed and tested. Our system transports whole secretions from the skin to an electrochemical LA biosensor using a continuous flow of phosphate buffered saline at the skin’s surface. The LA biosensor was fabricated by modifying screen printed carbon electrodes with osmium wired horseradish peroxidase and lactate oxidase. For continuous monitoring purposes, the microfluidic LA biosensor was connected with a flow cell made of polydimethylsiloxane (PDMS). The sampling device was fabricated by laminating a medical plaster on a PDMS flow-channel. Before the clinical tests, the characteristics of the LA biosensor were evaluated. Sufficient sensitivity (0.4232 nA/µM) and selectivity to LA (> 50fold against glucose, glutamic acid, phenylalanine, ammonia and ethanol) were confirmed. Besides, the medical plaster-based attachment contributed to the reduced leakage between the skin and the sampling device. Under the approval of the ethical committees, LA monitoring of patients with cardiovascular disease at the intensive care unit was carried out. As a result, temporal changes of sweat LA were successfully monitored.

1. Introduction
In clinical sites, vital signs such as heart rate and pulse are used in the treatments of severely dysfunctional patients. Temporal changes of those vital signs result in performing appropriate treatment because they reflect changes in physical condition. Kenzaka et al. reported that increased respiratory rate and shock index were correlated with the severity of illness in patients with sepsis [1]. Vital signs are mainly based on physical information for which measurement techniques are mature. On the other hand, chemical information, such as lactic acid and glucose, is also useful in assessing pathological conditions. Lactic acid (LA) is mainly produced in anaerobic metabolism and is released into the blood. Therefore, blood lactic acid (BLA) reflects oxygen metabolism in tissues [2–5]. Cocchi et al. reported that BLA correlated with in-hospital mortality [6]. For example, LA is used in the evaluation of sepsis, heart failure, liver disease, and lactic acidosis associated therewith [7]. Lactic acidosis is a condition in which BLA is 5 mM or more and the blood is acidic, and the mortality rate can be as high as 50% unless appropriate treatment is performed early [8–10]. Currently, BLA measurement requires regular blood sampling. Repeated blood sampling is a heavy burden for patients as well as it causes medical staff with extra work. Also, continuous measurement is difficult. Therefore, non-invasive and continuous LA measurement techniques that can be easily used even in clinical sites are required.
We focused on sweat, which is one of the biomarker-rich body fluids. Sweat is secreted from the sweat glands mainly for thermoregulation. There are 1.6 to 4 million sweat glands throughout the body, and their density varies depending on the body part [11, 12]. LA is also contained in sweat. Sweat LA (SLA) is produced by the metabolism of the sweat glands themselves, and the concentration of SLA is about ten times higher than that of BLA [13, 14]. Many of the previous literature represent that SLA mainly depends on the sweat rate; it also fluctuated for some other reasons such as the intensity of exercise [15–19]. However, the detailed kinetics of SLA secretion is not clarified. This can be associated with the fact that an appropriate technique for monitoring sweat contents has not been developed. In some previous studies, SLA was measured by collecting a large amount of sweat; thus it was likely that changes in SLA cannot be monitored under low sweating conditions. Hence, a simplified technique of sweat content monitoring, that is capable of use in clinical sites or in daily activities where it is difficult to collect a large amount of sweat, is strongly requested.

In this study, a non-invasive and continuous monitoring system for SLA was developed. The novelty of this system is that a small amount of lactic acid secreted from the skin's surface immediately dissolved in the career flow, and transported to a biosensor, which is specific to LA. We applied this system to patients under intensive care and verified the utility of SLA for evaluating oxygen metabolism in tissues. In this paper, we report on the design and characteristics of the microfluidic LA biosensor and the results of clinical trials.

2. Materials and methods

2.1. Construction of the sweat lactic acid monitoring system

The microfluidic SLA monitoring system (Figure 1) consists of an electrochemical LA biosensor, a sampling device with a flow channel and a tube pump (APSN23535RC, A&M, Japan). The sensor unit is connected to a potentiostat (ALS-701EX, CH Instruments Inc., USA). The LA biosensor measures LA as a change of the level of hydrogen peroxide generated by the enzymatic reaction of lactate oxidase (LOD). The production of hydrogen peroxide is converted into a redox current. The sampling device is used for collecting sweat contents. The system utilizes phosphate buffered saline (PBS) as a carrier flow. Whole secretions from the skin are dissolved in PBS and transported to the LA biosensor. Then, LA is selectively measured by the LA biosensor. PBS after the measurement is discharged as a waste liquid.

![Figure 1. Structure of the microfluidic SLA monitoring system.](image_url)

2.1.1. The microfluidic LA biosensor

The LA biosensor was formed by screen printing using carbon graphite paste. Figure 2 shows the structure of the LA biosensor. It has two working electrodes (WE), a counter electrode (CE) and a silver/silver chloride reference electrode (RE). The electrodes are covered with a polyvinyl chloride adhesive sheet having a microchannel in order to keep the sensing area
constant. Two WEs is coated with an Os-HRP redox polymer (002096, Bioanalytical Systems, USA).

In addition, LOD (T-47, Asahikasei Pharma Co., Japan) is immobilized on WE1. The microfluidic LA biosensor was fabricated by combining the LA biosensor with a flow cell made of polydimethylsiloxane (PDMS) with a microchannel (1 mm × 1 mm × 15 mm). The flow cell was prepared by pouring PDMS (SYLGARD184 Silicone Elastomer Kit, Dow Corning Corp., USA) into the flow channel mold made of polymethyl methacrylate (PMMA). In order to improve the durability against external impact, the biosensor was also installed in a PMMA casing as presented in Figure 3.

![Figure 2. Structure of the LA biosensor.](image)

![Figure 3. Appearance of the microfluidic LA biosensor.](image)

2.1.2. The sampling device

The sampling device was fabricated by combining a PDMS flow cell and a medical plaster (NSD3-2, NIPRO Co., Japan) with an elastic adhesive. The medical plaster was used to improve adhesion to the skin and prevent leakage of PBS during the measurement. The appearance of the sampling device is shown in Figure 4.

![Figure 4. Appearance of the sampling device.](image)

2.2. Characterization of the microfluidic LA biosensor

As shown in Figure 5, the experimental system for evaluating characteristics of the microfluidic LA biosensor was constructed by using a tube pump (APSN23535RC, A&M, Japan) and silicon tubes. The flow rate of the carrier flow was set to 50 µL/min. Cyclic voltammetry (CV) was performed under the presence of various concentrations of LA (0, 50, 100, 500, 1000 µM) in PBS. The sweep rate was 50 mV/s, and the sweep range was −0.1 to 0.6 V vs. Ag/AgCl. The driving potential of the biosensor was
determined based on the result of CV. Subsequent experiments were conducted under that potential. Constant potential amperometry (CPA) was performed to investigate the quantitative characteristic of the LA biosensor. The concentration of introduced LA solutions were 0, 10, 25, 50, 100, 200, 500, 1000 and 2000 µM. Then, the selectivity to LA was evaluated by introducing each solution (500 µM) of LA, glucose, glutamic acid, phenylalanine, ammonia, and ethanol into the system.

![Experimental system for evaluating characteristics of the microfluidic LA biosensor.](image)

**Figure 5.** Experimental system for evaluating characteristics of the microfluidic LA biosensor.

### 2.3. SLA measurement at the intensive care unit

This system was applied to SLA monitoring of patients with cardiovascular disease (approval of ethical committees: No. 3401 St. Marianna Univ. and No. 17-534 Meiji Univ.). The experiment was performed at the intensive care unit (ICU) of St. Marianna University School of Medicine Hospital. The sampling device was attached on the forearm of the patient. At the same time, sweat rate was also measured. Sweat rate was measured by using a micro sweat meter (TPL3520, Techno Next, Japan) near the sampling device. In addition, the part to which the sampling device was attached was sterilized with an alcohol sheet before the measurement.

### 3. Results and discussion

#### 3.1. Sensitivity and selectivity of the LA biosensor

Figure 6 shows the cyclic voltammograms obtained by introducing LA solutions with different concentrations to the LA biosensor. As shown in the figure, reduction currents (potential sweep in the negative direction) were almost similar within the potentials from 0.6 to 0.4 V vs. Ag/AgCl in any concentrations. In contrast, the output currents around 0 V vs. Ag/AgCl depended on the LA concentrations because of the redox reaction of Os-HRP. Therefore, the driving potential of the LA biosensor was determined to 0 V vs. Ag/AgCl. The operating potential was sufficiently low for reducing the influences of possible reductants in the body (e.g. uric acid, ascorbic acid, etc.). Figure 7 shows the calibration curve of the LA biosensor. The sensitivity of the LA sensor was 0.4232 nA/µM, and the calibration range was 25–500 µM. Although SLA is about 5–60 mM in normal cases, which exceeds the linear range, this calibration range is sufficient for the measurement. This can be associated with the mechanism of our system, which dissolves whole sweat into the carrier flow. SLA is diluted about 100-fold in the carrier flow. Table 1 shows the result of evaluating the selectivity of the LA biosensor. The responses to glucose, glutamic acid, phenylalanine, ammonia and ethanol were 2.04%, 1.78%, 1.72%, 1.80% and 1.69%, respectively, comparing with that of LA. That is, the selectivity was about 50-fold even for glucose with the lowest selectivity. Therefore, the biosensor possessed high selectivity to LA. From the above, it was confirmed that the LA biosensor was suitable for SLA measurement.
3.2. Real-time SLA monitoring at the ICU

SLA monitoring was also performed in the ICU. From the ethical point of view, the time of clinical test was limited to two hours at maximum. The system setting could be completed in about 2–3 minutes, which was appropriate for the use in regular operation. The medical plaster-based sampling device was responsible for reducing the time for attachment. Since the sampling device was tightly adhered to the skin, no peeling of the sampling device and no liquid leakage occurred throughout the experiment. Previously, the PDMS sampling device was attached directly to the skin and secured with an armband, which was time-consuming and resulted in frequent leakage. A typical result obtained from clinical tests is shown in Figure 8. Regardless of the sweat rate, it is possible to estimate the total secretion of LA because the flow rate and the area of the sweat sampling part are constant. The concentration of LA was calculated by dividing the secretion of LA by the sweat rate. As can be seen in the figure, LA secreted on the surface of the skin was monitored successfully. No noise was generated by the patient’s movements or the medical staffs’ work. These suggest that our system is useful for SLA evaluation. LA secreted from the skin may not only be produced at a constant rate but also depend on sweating. We will continue experiments using this system and investigate the dependence of SLA on sweating and the correlation between SLA and BLA. For clinical applications, we will also consider the appropriate sampling area and location.

|                | Lactic acid | Glucose | Glutamic acid | Phenylalanine | Ammonia | Ethanol |
|----------------|-------------|---------|---------------|---------------|---------|---------|
| Output (%)     | 100         | 2.04    | 1.78          | 1.72          | 1.80    | 1.69    |
| (n=5) Standard deviation | 0          | 0.25    | 0.31          | 0.37          | 0.41    | 0.23    |
4. Conclusions

We described the construction of the system for monitoring SLA to evaluate oxygen metabolism in tissues. The LA biosensor using LOD enabled specific detection of LA and had a sufficient response. This confirmed that the biosensor had the necessary performance for monitoring SLA on the skin surface, so it was decided to apply it to patients at the ICU. By using a medical plaster for the sampling device, it was able to adhere to the skin and measure without leakage. The SLA monitoring system was constructed by combining the LA biosensor and sampling device. As a result of conducting clinical tests using this system, we succeeded in real-time monitoring of SLA secreted from the surface of the skin. Although there are still many problems to be solved, practical application in clinical settings is expected in the future.

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