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Letter:

Wearable Biosensor Utilizing Chitosan Biopolymer for Uric Acid Monitoring

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A wearable biosensor was specifically engineered to measure uric acid, a biomarker present at wound sites. This biosensor, fabricated as a disposable and wearable device, was seamlessly integrated onto a polyethylene terephthalate (PET) substrate by utilizing carbon and silver conductive paste as the electrodes. The enzyme uricase was immobilized onto the working electrode by utilizing chitosan, a biocompatible material, to create this biosensor. Notably, the uric acid biosensor fabricated with chitosan showcased exceptional performance metrics, including remarkable output current values and impeccable stability. These findings suggest the prospective utilization of chitosan-based uric acid biosensors for the accurate measurement of uric acid on human skin in future applications.

Keywords: wearable biosensor, uric acid, uricase, chitosan

1. Introduction

Wearable biosensors leveraging the technology of microelectromechanical systems (MEMS) have been devised as a viable alternative to costly and intricate analytical apparatus in healthcare, offering compact and pervasive sensing solutions tailored for healthcare applications [1–4]. The seamless integration of biosensors into the Internet of Things ecosystem is pivotal to advance the frontiers of healthcare and medical monitoring, with the overarching goal of enhancing the quality of life (QoL) of individuals.

Numerous biosensors for application on the external surface of human skin have been devised. For instance, biosensors capable of detecting chemical compounds in sweat and gases in the skin derived from blood gases have been designed [5–9]. Among these, wound sensors that facilitate the monitoring of wound conditions have garnered significant interest. Chronic wounds significantly impact on the QoL of patients and impose a substantial financial burden owing to the recurring need for wound dressing replacement and prolonged hospital stays. Consequently, innovative wound care technologies, that offer simple monitoring, affordability, and wearability, with body-attachable sensors to fulfill these requirements are in high demand [10–12]. Therefore, a discernible call exists for the advancement of wound site treatment technologies at more affordable prices. Given the escalating costs associated with healthcare for patients and the ensuing implications on their QoL, sensors developed for this purpose must be not only cost-effective but also wearable for human use.

Research pertaining to the monitoring of wound sites has focused on techniques for measuring the chemical constituents generated at the site of injury, as well as physical parameters such as body temperature and extent of strain [13]. Among the various chemical components, uric acid (UA) has emerged as a noteworthy biomarker, owing to its robust correlation with wound severity as elucidated in previous studies [14]. Furthermore, UA levels are known to decrease owing to the action of uricase during bacterial infection. Consequently, UA has been employed as a reliable indicator to assess both the wound condition and infection status. Kassal et al. successfully fabricated a cutting-edge wound sensor by incorporating a Ag/AgCl reference electrode, Prussian blue-carbon working electrode, and counter electrode within a bandage while immobilizing glutaraldehyde and uricase [15]. Remarkably, this sensor can transmit real-time UA status to a computer or smartphone via near-field communication, making it a valuable tool for monitoring the dynamic status of wounds.

The aim of this study was to develop a wearable biosen-

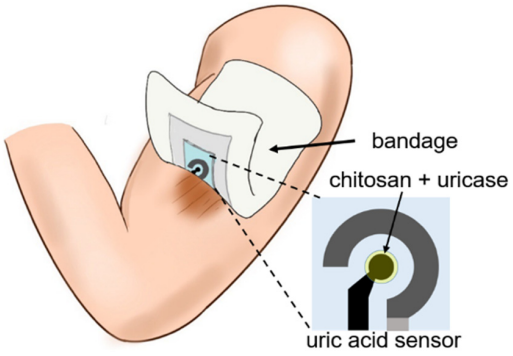


Fig. 1. Schematic diagram of a UA sensor worn on human skin for wound site monitoring.

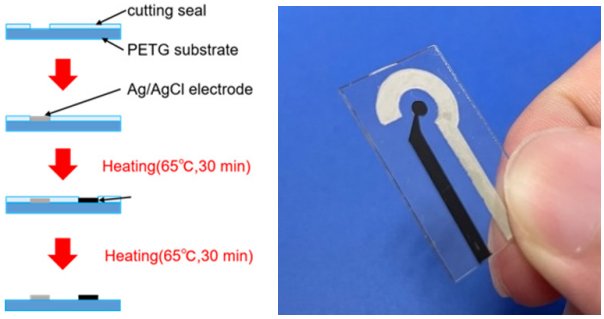


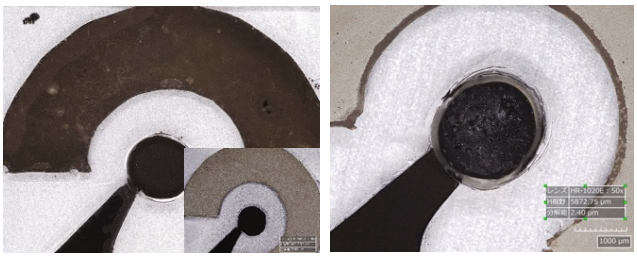
Fig. 2. Fabrication of UA biosensor and an image of fabricated UA biosensor on the PET substrate.

sor for wound site evaluation. Specifically, we fabricated a biosensor capable of measuring UA levels as an indicator of wound conditions. Our goal was to demonstrate a wound sensor by fabricating electrodes using conductive paint on a commercially available adhesive bandage, with uricase immobilized on the working electrode to enable biosensor functionality (**Fig. 1**). The catalytic reaction of hydrogen peroxide products resulting from the oxidation of UA allows the detection of the output current at lower potentials. In this letter, we present the response and sensitivity of a biosensor fabricated by immobilizing a biocompatible material chitosan for UA measurement.

2. Experimental Setup

2.1. Fabrication of Electrodes on a Polyethylene Terephthalate (PET) Sheet

Figure 2 shows the biosensor and fabrication procedure. First, a patterned sheet of electrodes fabricated using a cutting machine (GS-24, Roland) was attached to a PET substrate (1.5 mm × 2.5 mm, 1 mm thick). Second, a silver paste material (XA-3513, Fujikura Kasei Co., Ltd.) was spread by heating at 65°C for 30 min using a hot plate, and a paste material of carbon (XC-3050, Fujikura Kasei Co.) was spread as the working electrode using an electrode mold and fixed by heating at 65°C for 30 min using a hot plate. Finally, the patterned sheet was peeled



(a) (b)

Fig. 3. Image of UA biosensor. (a) Conparison of electrode appearance before (inner) and after chloride treatment. (b) Immobilization of uricase using chitosan on the carbon electrode.

off the substrate, and the fabricated silver electrode was chlorinated to form a silver chloride electrode.

A UA biosensor utilizing uricase is based on the specific enzymatic oxidation of UA by oxygen, resulting in the production of hydrogen peroxide, allantoin, and carbon dioxide. The reaction can be expressed as follows:

$$\mathrm{UA} + \mathrm{H_2O} + \mathrm{O_2} \xrightarrow{\text{uricase}} \text{Allantoin} + \mathrm{H_2O_2} + \mathrm{CO_2}.$$

The amperometric detection method can rely on the determination of the generated hydrogen peroxide.

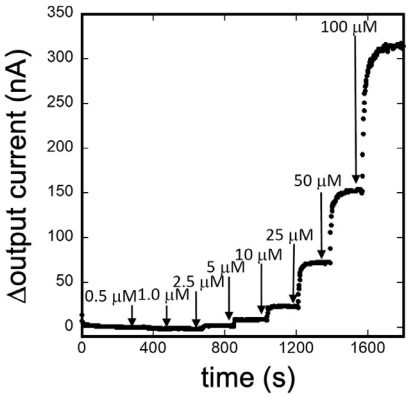
2.2. Fabrication and Measurement of UA Biosensor

Uricase was immobilized on a working electrode using chitosan, resulting in the fabrication of a UA sensor. Chitosan, known for its excellent biocompatibility, biodegradability, and non-toxicity, is widely utilized in biochemistry and medicine for applications such as wound healing, microneedles, hemodialysis membranes, and as a support for immobilized enzymes [16, 17].

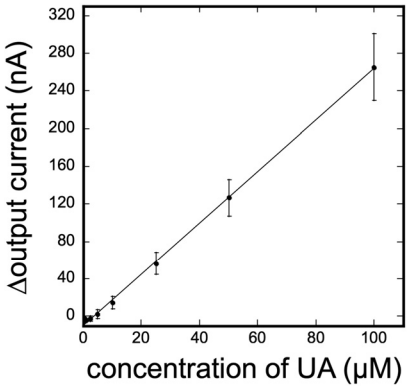
To prepare the UA sensor, a mixture of 10 mg of chitosan solution (3 wt%) and 1 mg of uricase (3.40 U/mg) was applied to the surface of the working electrode and dried in a refrigerator for 18 h. In the experimental setup, drops of UA solution ($C_6H_{12}O_6$, 5 mM) were added every 3 min to achieve varying concentrations of UA (500 nM to 100 mM) in a beaker containing 50 mL of phosphate buffer (PB, 50 mM, pH 7.0). The corresponding output current values were continuously measured by applying voltage of +0.8 V using potentiostat (EmStat4S, PalmSens). A calibration curve was constructed using output current values for each UA concentration.

3. Results and Discussions

Figure 3(a) presents a micrograph of a silver electrode that was prepared and subsequently treated with chloride, whereas **Fig. 3(b)** shows a magnified image of the working electrode after the immobilization of uricase using chitosan. Uricase was specifically immobilized onto the working electrode using a sheet stencil mask. The thicknesses of the carbon electrode, silver electrode, and



(a)



(b)

Fig. 4. (a) Temporal changes in the output of UA biosensor. (b) The calibration curve for UA biosensors.

chitosan film containing the enzyme measured 19.4 μm, 20.0 μm, and 6.1 μm, respectively, as evaluated with a contact-type thickness meter. An excellent response time was obtained for the UA biosensor by reducing the thickness of the chitosan film.

Figure 4(a) shows the response output of the fabricated UA biosensor, and **Fig. 4(b)** shows the calibration curve of the UA biosensor. The calibration curve was prepared using the average output current, allowing for quantification in the range of 500 nM to 100 μM. As the concentration of UA on the wound site is typically embedded within the range of 220 μM to 750 μM [18], this biosensor is suitable for measuring UA levels. Notably, the output current of the chitosan-based UA sensor at 100 μM was approximately four times that of our previous UA biosensor that utilized immobilization of a UV cross-linkable polymer.

4. Conclusion

In this study, we fabricated and evaluated a UA biosensor using chitosan as an enzyme-immobilizing material. The UA biosensor fabricated with chitosan exhibited superior performance than did conventional biosensors that utilize UV cross-linking agents for enzyme immobilization, in terms of output current value and stability. These findings suggest that the chitosan-based UA biosensor holds promise for measuring UA levels at wound sites. Further investigations should be conducted to optimize the amount and immobilization of uricase for the fabrication of a stable UA biosensor. By enabling the application of the fabricated biosensor to continuously measure the condition of a wound site, this study could reduce medical costs by decreasing the frequency of dressing changes and alleviating patient stress.

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