



## Soft contact lens biosensor for in situ monitoring of tear glucose as non-invasive blood sugar assessment

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### ABSTRACT

A contact lens (CL) biosensor for in situ monitoring of tear glucose was fabricated and tested. Biocompatible 2-methacryloyloxyethyl phosphorylcholine (MPC) polymer and polydimethyl siloxane (PDMS) were employed as the biosensor material. The biosensor consists of a flexible Pt working electrode and a Ag/AgCl reference/counter electrode, which were formed by micro-electro-mechanical systems (MEMS) technique. The electrode at the sensing region was modified with glucose oxidase (GOD). The CL biosensor showed a good relationship between the output current and glucose concentration in a range of 0.03–5.0 mM, with a correlation coefficient of 0.999. The calibration range covered the reported tear glucose concentrations in normal and diabetic patients. Also, the CL biosensor was applied to a rabbit for the purpose of tear glucose monitoring. The basal tear glucose was estimated to 0.11 mM. Also, the change of tear glucose induced by the change of blood sugar level was assessed by the oral glucose tolerance test. As a result, tear glucose level increased with a delay of 10 min from blood sugar level. The result showed that the CL biosensor is expected to provide further detailed information about the relationship between dynamics of blood glucose and tear glucose.

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### 1. Introduction

In the recent years, diabetes incidence is drastically increasing in the developed countries [1]. The main characteristic of diabetes is a chronically raised blood glucose concentration (hyperglycaemia) [2], which is a major risk factor for microvascular complications such as atherosclerosis, coronary artery disease, stroke, and peripheral vascular disease. Self-monitoring of blood glucose is widely used for maintaining appropriate blood glucose levels. This is usually performed by ‘finger prick’ test using a portable meter. Although the finger prick test is a nearly completed method, the glucose meter provides only a temporal value, and cannot follow

reflect the rapid change of blood glucose level (2.25 mg/dL/min) towards hypoglycaemic and hyperglycaemic events [3]. Therefore, continuous glucose monitoring method which does not become restriction of daily life is strongly requested.

A glucose sensor for continuous blood sugar monitoring is one of the most approach and they are also expected to improve public health and reduce total medical costs. Since the first glucose sensor was constructed by Clark and Lyons in 1962 [4], numbers of glucose sensors have been developed by many different approaches, such as electrochemical [5–8], spectrophotometric [9,10] and fluorescence methods [11,12]. With the purpose of continuous blood glucose monitoring for automatic glucose control, numbers of implantable biosensors used in subcutaneous tissue and vessel was reported [13–21]. For instance, a miniaturized implantable needle-type glucose sensor associated with an artificial endocrine pancreas system, which consists of a microcomputer and a 2-syringe driving system [19,20]. Although these sensors provide accurate blood sugar level, the implant process requires a surgical operation even if that is a minimal invasive surgery. It would bring some mental and physi-

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cal sufferings to diabetic patients due to the obstructions in daily life.

On the other hand, several information and communication technologies suitable for constant use in biomonitoring purpose have been reported [22,23]. Particularly, short-range wireless communication (including body-wired communication (BWC)) so-called body-area network (BAN) is a promising technique for this purpose. BAN and related technologies enables to connect multiple 'wearable' biomonitoring sensors each other, and transmit the signals to a portable control system like a mobile phone. The users can benefit an appropriate feedback in real-time [24]. A non-invasive and continuous glucose monitor (CGM) suitable for such a BAN-related technologies are thus strongly requested. Generally, blood glucose level can be estimated non-invasively by monitoring physiological fluids that reflect blood glucose levels, such as urine, tear, mucus, sweat, saliva, etc. Particularly, we focused on glucose in tear fluids. Increase of tear glucose in hyperglycemia was first reported in 1930s [25] and a definite relationship between tear glucose and blood glucose was also reported [26,27]. Recently, an advanced study on tear glucose before and after administration of a carbohydrate load was carried out by Lane et al. Their result indicated that a correlation between tear glucose and blood glucose concentrations can be found in both diabetic and normal subjects [28].

Continuous blood sugar assessment with a BAN-friendly device in mind, we have designed and fabricated a contact lens (CL) biosensor that measures tear glucose continuously. The final goal of our device includes integration of a power source, a BWC transmitter, an electrical circuit and a sensing probe. The biosensor was fabricated using micro-electro-mechanical systems (MEMS) technique to form electrodes on the peripheral surface of a polydimethyl siloxane (PDMS) contact lens. In this paper, we report details of the structure and fabrication of the CL glucose sensor. And the basal tear glucose concentration of the rabbit is estimated using the sensor. Also, dynamic change of tear glucose level is monitored and compared with change of blood glucose level.

## 2. Experimental

### 2.1. Materials

D(+)-Glucose (Wako Pure Chemical Industries Ltd., Japan) was used to prepare standard glucose solution. Physiological sodium chloride solution (OTSUKA Pharmaceutical Co., Ltd., Japan) was prepared to clean the biosensor in animal experiment. Glucose oxidase (GOD: EC 1.1.3.4, 146,000 units/g) was purchased from Sigma-Aldrich Company of USA. Hydrophobic PDMS (Silpot 184, Dow Corning Toray Co., Ltd., Japan) was used as the substrate material of the biosensor. PMEHL was obtained by copolymerizing 2-methacryloyloxyethyl phosphorylcholine (MPC) with 2-ethylhexyl methacrylate (EHMA) (MPC:EHMA=3:7) at 60 °C for 2 h. A phosphate buffer solution (PB: pH 7.4, 20 mM) was prepared for evaluation of the sensor characteristics.

### 2.2. Design of CL glucose sensor

The CL biosensor was designed for in situ monitoring of tear glucose level. PDMS was used as the body material of the sensor. The biosensor has a flexible hydrogen peroxide electrode (Pt working and Ag/AgCl reference/counter electrodes), which was formed on a thin PDMS membrane (size of 3 mm × 45 mm, thickness: 70 μm). For the purpose of application on eye site, the biosensor utilized a soft PDMS contact lens (base curve radius: 8.6) as a support of the flexible electrode. The output signal of the CL biosensor was tentatively read using a wired line in the preclinical experiment and tested that the sensor works while it is worn on the eye.

### 2.3. Fabrication and evaluation of the CL biosensor

Fig. 11 shows the fabrication process of the flexible electrode. At first, a 70 μm thick PDMS membrane was formed onto a 525 μm thick dummy silicon wafer by spin-coating system (1H-DX II, Mikasa Corp., Tokyo, Japan) and cured at 80 °C for 30 min. A 200 nm thick Pt film was sputtered onto the PDMS membrane by a sputtering system (EIS-220, Elionix Co., Ltd., Japan) via a titanium stencil (thickness: 40 μm), which is fabricated by a laser microfabrication system (MD-V9610, KEYENCE Corp., Japan). The reference electrode was formed as a bilayer of a 300 nm thick Ag film and a 200 nm Pt for the purpose of improving adhesion. Then, the PDMS membrane with the electrode was released from the dummy wafer. The electrodes were covered with a thin insulating PDMS membrane except the sensing region and the electrode terminal region. After that, the Ag/AgCl electrode at the sensing region was electrochemically chloridized with 1.0 mM hydrochloric acid solution by applying a constant voltage of −60 mV. Thus, the flexible electrode was obtained.

The CL biosensor was constructed by following steps. A soft PDMS contact lens was formed by molding process. The PDMS resin was degassed with a vacuum chamber for 30 min and cured at room temperature for 24 h. As shown in Fig. 11a, the flexible electrode was bonded onto the peripheral surface of the CL using PDMS as a binder. Then, a 1.0 μL mixture of GOD (0.523 mg) and 10 wt% PMEHL solution was spread onto the sensing region of the sensor and cured for 3 h at 4 °C (Fig. 11b). The CL biosensor thus was obtained using biocompatible polymer.

In vitro characterization was carried out using a batch measurement system. The sensing region of the biosensor was placed in a 20 mL measuring cell filled with PB (pH 7.4, 20 mM). The electrode terminal was connected to a potentiostat (MODEL 1112, Fuso Inc., Japan). A constant voltage of +400 mV versus Ag/AgCl reference/counter electrode was applied to Pt working electrode. The output current induced was recorded using a computer via 16 bit A/D converter (ADC-16, pico Technology Ltd., UK).

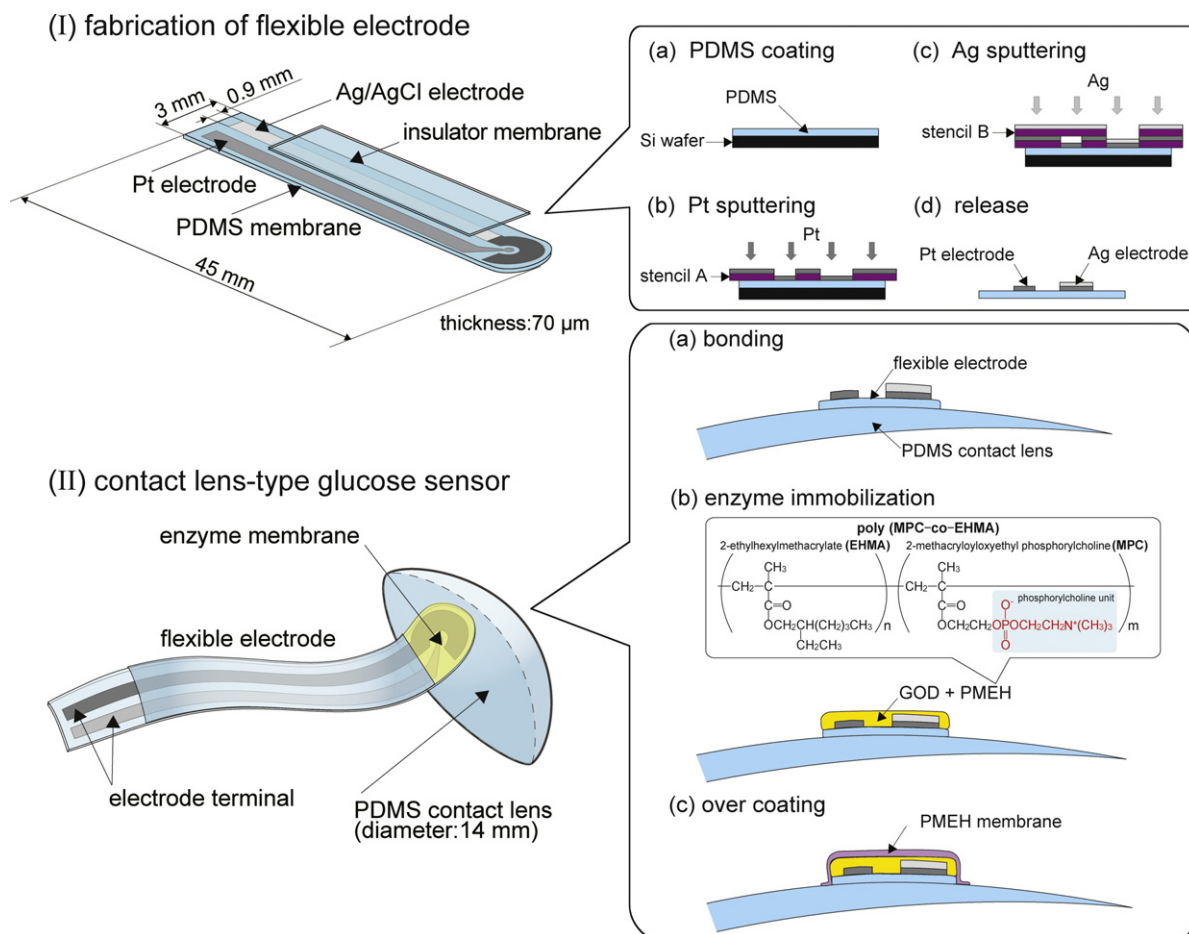
In order to prevent enzyme leakage at the GOD-modified electrode, the enzyme membrane was overcoated with a 1.0 μL PMEHL solution (Fig. 11c). An optimal concentration of PMEHL was defined by evaluating the characteristics of the biosensors with overcoating with various concentration of PMEHL (3, 5, 10 wt%).

### 2.4. Continuous monitoring of tear glucose using the CL biosensor

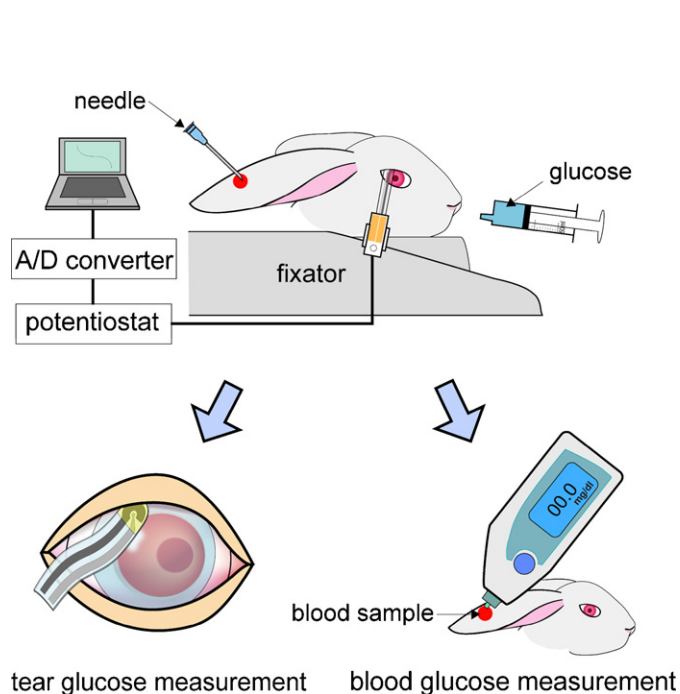
Preclinical ocular tear monitoring test with the CL biosensor was carried out on a rabbit (Japan white rabbit, age: 55 months, weight: 3.9 kg). As a first step, the basal tear glucose level was measured. The rabbit was fixed using a cylindrical fixation device without any anesthetic. The CL biosensor was cleaned with physiological sodium chloride solution and then attached on the eyeball of the rabbit. After that, the biosensor was connected to a potentiostat, and a constant potential of +400 mV (vs. Ag/AgCl) was applied to the Pt electrode.

The dynamics of tear content due to tear secretion was also monitored with the CL biosensor. After a stable period as well as the above mentioned experiment, 0.5 mM glucose (50 μL) was dropped into the eye of the rabbit. The change of the output current was recorded and analyzed by the computer.

Finally, the change of tear glucose level induced by the change of blood glucose level was monitored. As shown in Fig. 2, the CL biosensor was worn on the eyeball of the rabbit for continuous monitoring of tear glucose, and glucose solution was then orally administrated to the rabbit (1 g of glucose per 1 kg of weight). At the same time, the blood glucose level was measured using a self glucose monitoring kit (MEDISAFE, TERUMO Co., Japan). The blood sample was collected from the helix vein of the rabbit with



**Fig. 1.** Fabrication method of the CL biosensor. (I) The flexible electrodes (a 200 nm thick Pt working electrode and a 300 nm thick Ag/AgCl counter/reference electrode) were formed onto a 70  $\mu\text{m}$  thick PDMS membrane; (II) the flexible electrodes were bonded onto the surface of the PDMS contact lens using PDMS and then GOD was immobilized using PME on the sensing region of the electrodes. Finally, the enzyme membrane was overcoated by PME.



**Fig. 2.** Measurement method of tear glucose concentration with the CL biosensor. Blood glucose level was measured by a commercially blood glucose monitoring kit, simultaneously.

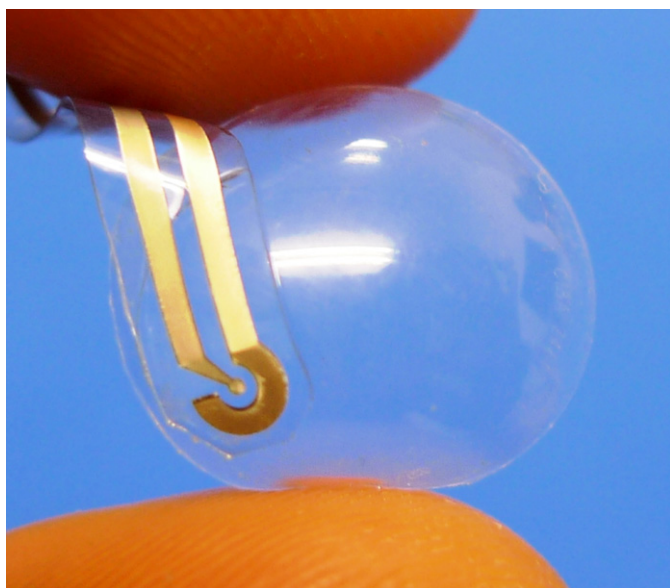
a syringe needle at before glucose administration, 0, 15, 30, 45, 60 min, respectively. (The animal experiment is approval number: 0110211B, Tokyo Medical and Dental University Animal Experiment Committee.)

### 3. Results and discussions

#### 3.1. Characteristics of CL biosensor

Fig. 3 shows the appearance of the soft CL biosensor. Using PDMS of form the soft CL, the sensor had a suitable flexibility for wearing applications. The film electrode showed high adhesion to the PDMS substrate and did not crack or peel off after bending. Also, no strain of deformation due to the stress of electrode deposition was confirmed.

As a result of in vitro characterization, the biosensor showed a rapid response to glucose (Fig. 4). According to the result, the output current was related to the glucose concentration from 0.03 to 5.0 mM and the calibration range included normal tear glucose level of humans (0.14 mM). The sensor was thus expected to be useful for in situ monitoring of tear glucose. However, the characteristics of the biosensor (no PME-coating at the sensing region) significantly degraded after application on the rabbit (Fig. 5). In order to solve this problem, the enzyme membrane was coated with additional PME membrane to prevent enzyme leakage. The effect of in the additional PME is shown in Fig. 6. As the figure indicates, the output current decreased as the concentration of the PME solu-



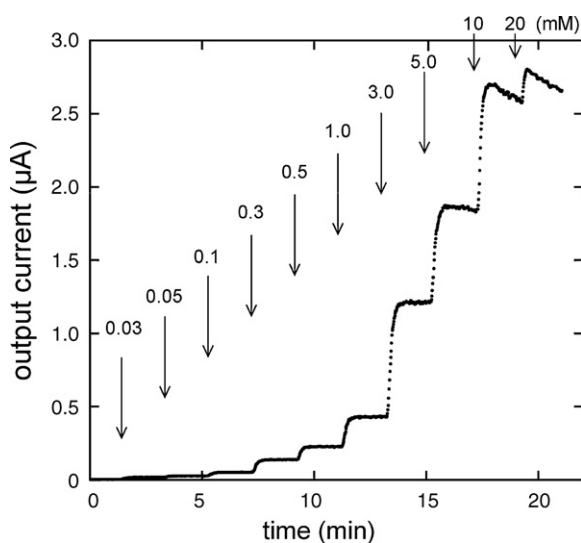
**Fig. 3.** Photograph of the CL biosensor constructed by biocompatible materials (PDMS and hydrophilic PME) and the flexible electrodes (Pt working and Ag/AgCl counter/reference film electrodes formed onto the PMDS membrane).

tion increased. This is because the additional membrane decreased not only the mobility of entrapped enzyme, but also decreased the permeability of the other chemicals to be measured. However, the repeatability of the biosensors with PME-coating increased by this improvement. Particularly, coating with 3% PME showed optimal trade-off in the sensitivity and the reproducibility. Based on above results, 3% of PME was defined as optimal concentration. The small box of Fig. 6 shows the calibration curves of the biosensor with 3% PME-coating before and after application. The output currents are represented by the following equations:

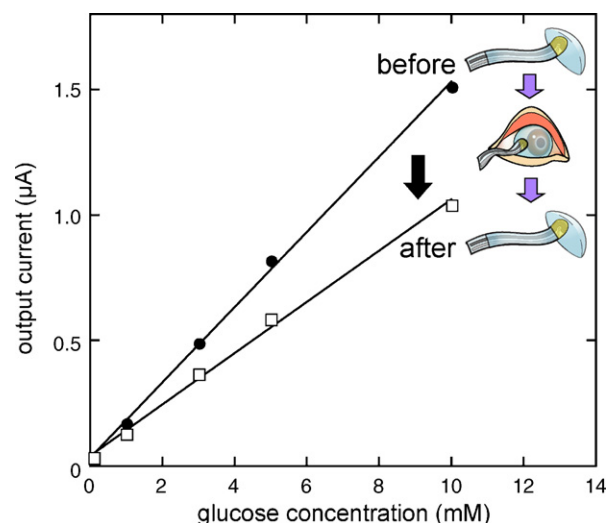
before application:

$$\text{output current } (\mu\text{A}) = 0.245 \times [\text{glucose (mmol L}^{-1})]^{0.831}$$

(coefficient of correlation of 0.999) (1)



**Fig. 4.** Typical response of the CL biosensor with no PME-coating to glucose concentration in vitro (constant voltage of +400 mV vs. Ag/AgCl, pH 7.4, 20 mM phosphate buffer solution). The output current increased immediately after the glucose solution was dropped into PBS.



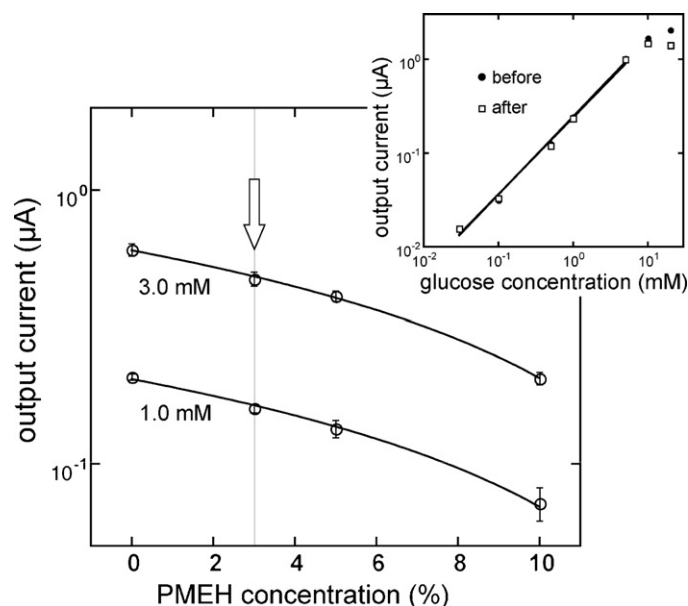
**Fig. 5.** Comparison of the calibration curves before and after application to the rabbit's eye. (●) Before application; (□) after application.

after application:

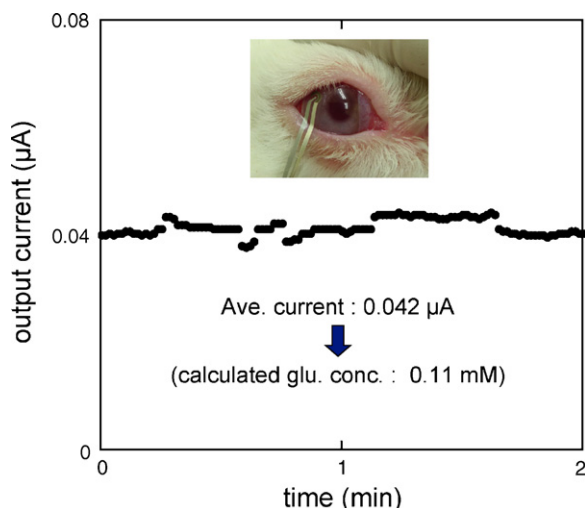
$$\text{output current } (\mu\text{A}) = 0.239 \times [\text{glucose (mmol L}^{-1})]^{0.818}$$

(coefficient of correlation of 0.999) (2)

In both cases, the calibration ranges were 0.03–5.0 mM as well as the older version without additional coating. The characteristics of the improved sensor did not change after application on the eye of the rabbit. From this result, the soft CL biosensor with sufficient wearability and durability for ocular biomonitoring was successfully obtained.



**Fig. 6.** Output current variation caused by a coating concentration of PME (no PME, 3%, 5%, 10%) 1.0 and 3.0 mM glucose. The largest current was shown in case of no PME-coating. The current decreased with the increasing of PME concentration. Inset box is calibration curves of the biosensor before and after application. (●) Before application; (□) after application.

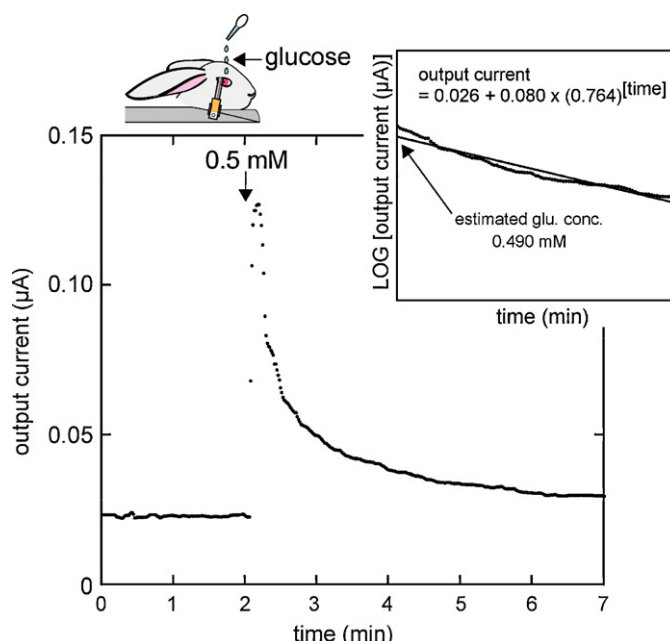


**Fig. 7.** Tear glucose monitoring using the CL biosensor on eye site. A stable output current was observed and the mean output current value was 0.042  $\mu\text{A}$  (The basal tear glucose concentration of the rabbit was estimated to 0.11 mM).

### 3.2. Tear glucose monitoring with CL biosensor

In situ ocular biomonitoring of tear glucose was finally taken place. Fig. 7 shows a typical response of the CL biosensor worn to the eye of the rabbit. As shown in the figure, stable current was observed and the mean value was 0.042  $\mu\text{A}$ . According to Eq. (1), the basal tear glucose level of the rabbit was estimated to be 0.11 mM, which was almost consistent with previous reports of humans [29–36,28,37,38]. Generally, tear glucose level can be affected by mechanical and chemical irritations. Considering the stable current found in this experiment, irritation was considered to be minimized due to the CL-like body as well as the previous report by Asher et al. [39,40]. The result indicated that the biosensor well worked at the steady and settled state. In the present stage, biochemical components in tear fluids such as ascorbic acid and the other proteins could influence output current of the biosensor. This can be improved by applying some selective membranes. On the other hand, the biosensor measures glucose concentrations as changes of hydrogen peroxide concentrations induced by enzymatic reactions. The impact of peroxide formation on eye irritation has to be considered. Peroxide formation as one of reactive oxygen radicals, as well as superoxide anion and the hydroxyl radical presents in human tears [41]. In order to inhibit the activities of those oxygen radicals and protect ocular tissues, tears also include several enzymes and antioxidants such as superoxide dismutase, catalase, glutathione, glutathione peroxidase, tocopherol and ascorbic acid. They catalyze or directly join those reductions of the oxygen radicals. Wilson et al. also reported that  $\text{H}_2\text{O}_2$  is rapidly eliminated on the ocular surface, chiefly by enzyme activity of the conjunctiva and cornea [42]. Thus, these evidences demonstrate that there is no affect of peroxide formation on eye irritation.

Dynamics of the tear content by tear secretion was also measured. Fig. 8 shows the response of the CL biosensor to instillation of standard glucose solution. The output current immediately increased when the standard glucose solution was dropped and decreased gradually to the initial value. The curve of the current down to initial state indicates the process of replacement of standard glucose with the fresh tear fluids secreted from lacrimal gland. The small box of Fig. 8 shows a semi-log plot of the current and the regression curve derived from the following equation as a



**Fig. 8.** Temporal change of the output current by eye drop of 0.5 mM glucose solution. The current rapidly increased until a signal peak following instillation of glucose solution, and then returned to the initial level, gradually.

function of time.

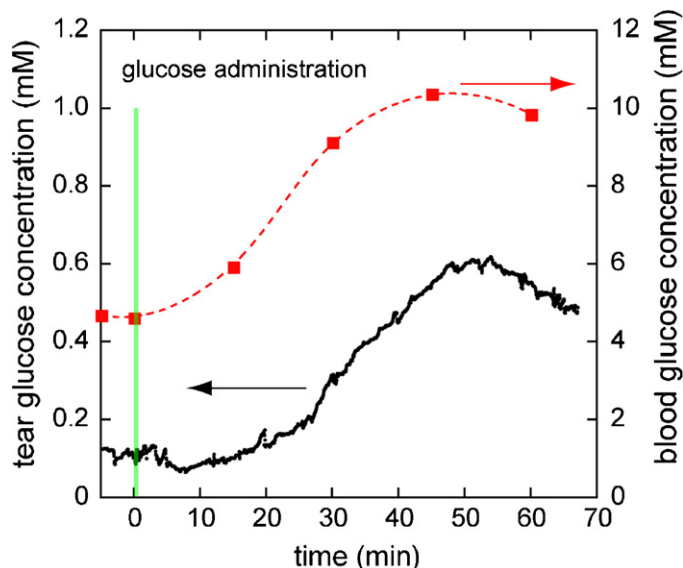
$$\text{output current } (\mu\text{A}) = 0.026 + 0.080 \times 0.764^{\text{time (min)}}$$

(coefficient of correlation of 0.974)

(3)

The estimated glucose concentration was 0.49 mM by Eq. (3). The estimated concentration (0.49 mM) is nearly equal to the concentration of the dropped glucose (0.5 mM). Considering the presence of basal tear, this value stands to reason. The results indicated that the CL biosensor was also useful in monitoring of dynamics of tear glucose on eye site.

Based on above results, change of tear glucose induced by change of blood glucose was also monitored using the CL biosensor. Fig. 9 shows a typical tear dynamics related to elevation of blood



**Fig. 9.** Chronological change both in tear glucose level and blood glucose level. The tear glucose level increased with a delay of 15–20 min from blood glucose level.

sugar level induced by oral administration of glucose. The initial tear glucose level was estimated to be approximately 0.116 mM, which was almost similar to the previous result. After oral administration of glucose, the blood glucose level increased immediately, and reached a peak after 45 min. The tear glucose level followed it with a delay of approximately 10 min from the blood sugar level and reached to the peak after 55 min. After that, the tear glucose level decreased gradually. The highest tear glucose concentration was estimated to 0.61 mM by Eq. (3). The tear glucose increased by approximately six folds (0.116–0.61 mM) while the blood sugar level increased by two folds. This result is consistent with the previous reports of humans [28]. Since the deference of metabolic capacities between humans and rabbits, it is possible that variation of the increasing rate differs from that of rabbits. However, the tear glucose level was successfully monitored ‘continuously’ with the CL biosensor in real-time. Tear glucose also changes by individual variation and daily fluctuation (including physiological condition) [28,40]. In the previous studies, these fluctuations were measured by analytical method including tear sampling or discrete method. The in situ monitoring by the CL biosensor is expected to provide further detailed information about the relationship between dynamics of blood glucose and tear glucose.

Abnormal findings such as inflammation were not observed after the test. Despite presence of the read-out wiring, the rabbit was settled through the experiment. Tear content can be thus measured non-invasively and non-restrained. These are the essential characteristics for next generation bioinstrumentation systems such as BAN-based healthcare sensors. Since the sensor body was formed with PDMS, some kinds of microchips can be implanted in the CL body. The other components like transmitters can be thus integrated by miniaturization of each component. Moreover, the biosensor is useful for other chemical substances by choosing an adequate enzyme. Also, the biosensor is not only useful in ocular biomonitoring, but also useful in chemical sensing on any surface of the human body because of the soft and flexible structure.

#### 4. Conclusions

In this work, the CL biosensor for continuous monitoring of tear glucose was fabricated and tested. GOD modified electrode was formed on a PDMS contact lens using MEMS techniques. Owing to the wearable structure of the biosensor like a contact lens, non-invasive ocular biomonitoring was established with minimum irritations. As a result of in vitro characterization, the biosensor had a rapid response to glucose and appropriate calibration range (0.03–5.0 mM), which included the reported tear glucose levels. In the preclinical animal test, the biosensor successfully worked both in temporal measurement of tear glucose and continuous monitoring of tear dynamics. According to the oral glucose tolerance test, the tear glucose changed with a delay of 10 min from the blood sugar level. The rabbit was sufficiently settled during the experiment because of the wearable structure. Thus, non-invasive ocular biomonitoring was successfully established using the CL biosensor. Although there are still several struggle points, an advanced blood sugar assessment can be thus expected.

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