analytical chemistry

pubs.acs.org/ac Article

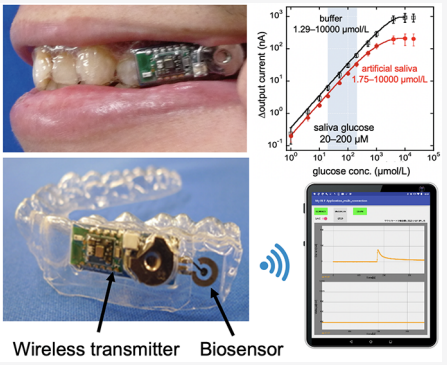
A Wearable Cellulose Acetate-Coated Mouthguard Biosensor for In Vivo Salivary Glucose Measurement

Takahiro Arakawa,* Keisuke Tomoto, Hiroki Nitta, Koji Toma, Shuhei Takeuchi, Toshiaki Sekita, Shunsuke Minakuchi, and Kohji Mitsubayashi*

Cite This: https://dx.doi.org/10.1021/acs.analchem.0c01201 Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: In this study, a cellulose acetate (CA) membrane is formed as an interference rejection membrane on a glucose sensor to measure glucose in saliva. Glucose in saliva is successfully measured in vivo without any pretreatment of human saliva. A mouthguard (MG) glucose sensor is developed to monitor salivary glucose, which is reported to be correlated with the blood glucose level. Salivary components of ascorbic acid (AA) and uric acid (UA) hinder the accurate measurement of the glucose concentration of human saliva. CA-coated electrodes are prepared to investigate the interference rejection membrane. To measure hydrogen peroxide, which is a reaction product of glucose oxidase, effects of AA and UA are examined. Characteristics of the fabricated biosensor are examined on the basis of artificial saliva. The as-developed MG sensor can quantify the glucose concentration in the range of 1.75−10 000 μmol/L, which includes a salivary sugar concentration of 20−200 μmol/L. For the measurement of saliva samples collected from healthy subjects, the output corresponding to the concentration is confirmed; this suggests the possibility of glucose measurement. This MG glucose sensor can provide a useful method for the unrestricted and noninvasive monitoring of saliva glucose for the management of diabetes patients.



In recent years, there has been an increase in the number of people suffering from lifestyle-related diseases such as hypertension, dyslipidemia, and hyperuricemia has increased. In particular,[1,2] 420 million people worldwide suffer from diabetes, already reaching 3.165 million in Japan.[3,4] Diabetes is a chronic hyperglycemic condition that can cause complications such as retinopathy, nephropathy, and neuropathy, and it requires appropriate management of blood glucose levels.[5,6] In general, self-monitoring of blood glucose (SMBG) is traditionally invasive, and it is most commonly performed with finger-prick testing using a blood glucose meter.[7] However, there are many problems of physical and mental burden and risk of infection for diabetes patients. Moreover, circadian fluctuations such as postprandial hyperglycemia and nocturnal hypoglycemia have been reported.[8,9] Therefore, methods for a new noninvasive and continuous evaluation of blood glucose level are strongly required.

A noninvasive method for evaluating blood sugar and for measuring body fluid components correlated with blood sugar has been reported.[10−15] A method for measuring glucose in interstitial fluid, which is done with a continuous blood glucose meter, is already in the market; however, it necessitates puncture, as in the case of self-measurement of blood glucose.[16] Urine sugar measurement is easy to sample, but it is unsuitable for continuous monitoring.[17]

Sweat is one of the biological fluids that can be easily sampled. The eccrine gland, which is the source of its discharge, is distributed throughout the body, especially in the hands, feet, lower back, and under the arms.[18−21] Sweat has been used for the evaluation of diseases such as cystic fibrosis, and it has attracted attention as a source of information on hydration status and medication as the demand for health monitoring devices has increased. In recent years, sweat has been utilized for the detection of disease markers such as sodium, potassium, calcium, phosphate, and glucose.[22] Sweat glucose levels have been reported to be 0.06−0.11 mmol/L for healthy subjects and 0.01−1 mmol/L for diabetics.[12] Wearable devices for measuring sweat glucose concentration in various forms have been reported.[23] Wang et al. have developed a spectacle-type sweat-glucose-concentration-measuring device.[24−26] This device can measure the glucose concentration in sweat with a glucose-sensitive pad connected to the nasal bridge pad.

Chu et al. developed a soft contact lens (SCL)-type tear-sugar sensor using polydimethylsiloxane (PDMS) as a substrate material, and realized stable measurement of tear glucose from the eye of a rabbit.[27,28] They was reported the comparison of the time-series change of tear sugar at the time

Received: March 19, 2020
Accepted: July 23, 2020

of oral ingestion of glucose was compared with the blood glucose level with a delay of 8 min.

On the other hand, saliva contains glucose at a concentration of about 1/100 of the concentration of blood glucose, which is secreted continuously at about 0.56 mL/min.[29,30] In addition, the oral cavity has a space where sensor devices can be attached easily. Mouthguard (MG) sensors for measurement of glucose, lactate, and uric acid in saliva have already been reported.[31−33] There is a report on a biosensor that can temporarily collect saliva externally and measure it.[34] Some microfluidic-paper-based devices for salivary analysis have been studied to be used as diagnostic tools.[35,36] Though many methods for sampling and measuring saliva have been published, direct monitoring of saliva glucose concentration in an oral cavity without pretreatment has not been reported.

In previous research, an MG glucose sensor was developed for the measurement of salivary glucose concentration, however, contamination of saliva has been found to be a problem, and it has not been measured in the human oral cavity.[32] In this study, we investigated the interference rejection membrane, which improved the reaction of ascorbic acid (AA) and uric acid (UA) in saliva on electrodes of MG. Futhermore, we developed a measurement application using a mobile terminal, and examined the continuous measurement of salivary glucose concentration.

EXPERIMENTAL METHODS

Measurement of Glucose Using Glucose Oxidase. We chose the enzyme of glucose oxidase (GOD) for recognition of glucose in saliva. A GOD mixture was applied to the sensing region of the working electrode. GOD can catalyze glucose in the presence of oxygen, producing gluconolactone and hydrogen peroxide at the working electrode. Through the following enzymatic reaction on the working electrode:

$$\text{glucose} + O_2 \xrightarrow{\text{GOD}} \text{gluconolactone} + H_2O_2 \quad (1)$$

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

The output current was monitored using a potentiostat and galvanostat machine (model 1112, Fuso Co. Ltd.). In our developed sensor system, the hydrogen peroxide produced by the catalytic reaction of GOD immobilized on the electrode, can be detected by the constant potential−current measurement using a wireless module integrated with a potentiostat. Therefore, the glucose concentration in saliva can be continuously measured.

Fabrication of the Electrode Sensor. Polyethylene terephthalate glycol (PETG, Erkodent Erich Kopp GmbH) was chosen as a MG material to form the supporting structure. This MG material of PETG and electrodes of Pt and Ag are biocompatible for human use, and their safety is compensated.[37,38] In addition, the PETG substrate shows good adhesion of sputtered Pt and Ag. A schematic image of the MG biosensor is shown in Figure 1a. A working electrode and a counter/reference electrode of the glucose sensor consisted of 200 nm thickness of Pt electrode (1.6 mm^2 size) and 300 nm thickness of Ag electrode (16 mm^2) that had been coated using a parallel-plate sputtering machine (Canon Anelva Co.). The other parts of the electrodes were covered with polydimethylsiloxane (PDMS) and insulated. In order to insulate the electrodes, the base and catalyst of Silpot 184 PDMS (Toray Dow Corning Co., Ltd.) were mixed at 9:1 ratio

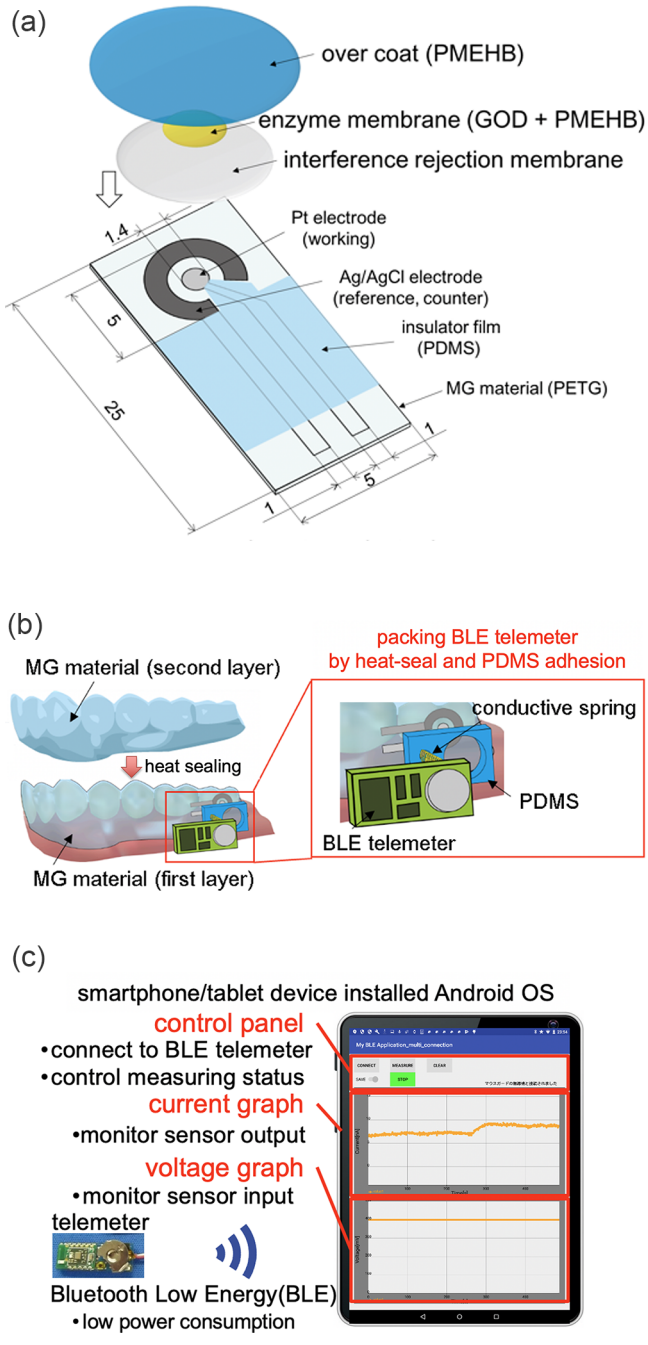


Figure 1. Schematic of the MG glucose sensor and measurement system. (a) Schematic of the glucose sensor. The sensor consists of an interference rejection membrane and enzyme membrane on Pt and Ag/AgCl electrodes. (b) Mounting of MG-type sensor using conductive spring and PDMS sheet. (c) Portable wireless measurement system using Android devices.

and deaerated for 2 min with a planetary centrifugal mixer. The PDMS mixture was spread and left to dry on a hot plate at 60 °C for 1 h. Finally, the Ag electrode was chloridized to form the Ag/AgCl electrode using electrochemical treatment of 0.1 mmol/L hydrochloric acid solution.

For immobilization of glucose oxidase (GOD, Wako), poly(MPC-*co*-EHMA-*co*-MBP)(PMEHB) with excellent biocompatibility and high mechanical strength was used. GOD was mixed with a PMEHB solution (5 wt % in ethanol), and an enzyme solution was added on the Pt electrode coated with a

https://dx.doi.org/10.1021/acs.analchem.0c01201
Anal. Chem. XXXX, XXX, XXX−XXX

contaminant-suppression membrane, and GOD was comprehensively immobilized by cross-linking by UV irradiation.[39] In order to reduce the leakage of the enzyme and the adsorption of proteins in saliva to the Ag/AgCl electrode, the electrode sensitive part was overcoated with PMEHB (1 wt % in ethanol). GOD mixture was also prepared prior to application by mixing GOD from *Aspergillus niger* (EC 1.1.3.4, 900 units/g, Sigma-Aldrich Co.) with 10 wt % PMEHB at 1 mg:10 μL ratio using a vortex. To produce electrode sensors capable of glucose measurement, GOD and PMEHB were coated on the working electrode. Finally, the sensing region of each electrode was rinsed by immersion in phosphate buffer solution (PBS) for 30 min. Thereafter, the electrode sensors were rinsed with distilled water, dried, and stored.

Fabrication of the MG Glucose Sensor. Fabrication of the MG glucose sensor involved production of a dental cast, vacuum forming of the PETG MG support, electrode sensor fabrication on the MG support, wireless transmitter installation, and packaging (Figure 1b). Details of fundamental fabrication of MG was described in a previous study.[32,40] Previous methods have reported the fabrication of MG biosensors using thermal welding. However, the welding of Pt and Ag electrode parts with PETG caused disconnection of electrodes due to thermal damage. In this study, the electrode area was sealed by joining the vicinity of the electrode with PDMS, in addition to thermal welding, and the waterproofness of the part was examined. The adhesion by PDMS was evaluated on the basis of tensile shear bond strength test. In the adhesion evaluation, a test piece in which two layers of MG materials were bonded to each other with a length of 5 mm using PDMS was placed in a tensile/compression tester (SV-55C, Imada Seisakusho) and pulled at a tensile speed of 45 mm/min. The bond strength was calculated and evaluated from the maximum load (N/15 mm).

In order to save the space between the connection terminal of the wireless module and the electrode, the connection between the electrodes and the wireless module circuit used a conductive spring with nickel plating (⌀, 1.0 mm; L, 3 mm; spring constant). In the manufacture of the MG biosensor, first a gypsum model of the subject's upper jaw (or lower jaw) dentition was first prepared, and this gypsum model was installed in a suction-type molding machine (no. 101, Yamahachi Dental Mfg). MG was three-dimensionally molded by suction molding after heat softening the material. Next, two electrodes were formed on the MG by sputtering, followed by PDMS insulation treatment, hydrochloric acid chloride treatment, and CA membrane coating. Next, after fixing a Bluetooth low energy (BLE)-type wireless measuring instrument was fixed to a sheet coated with PDMS solution in the vicinity of the electrode sensitive part, and the second MG was stacked from the top and hermetically mounted by heat welding. Thereafter, the MG was heated at 60 °C for 2 h to solidify PDMS to seal and waterproof the vicinity of the electrode. Finally, the sensor sensitive part was exposed, and then an MG biosensor was formed with enzyme immobilization.

The conventional wireless measurement system of the MG sensor requires a personal computer environment with a BLE receiver connected via USB, which limits the range of movement for the MG sensor. Since many mobile devices equipped with Android OS have BLE function, we developed an application for Android OS (Figure 1c). The application includes functions for searching around the wireless measuring instrument for MG sensors, receiving data, graphing, and storing data.

For measurement of glucose using MG biosensor, GOD catalyzes the oxidation of glucose in the presence of oxygen, to produce gluconolactone and hydrogen peroxide at the working electrode through the enzymatic reaction of GOD. The output current produced can be measured by amperometry, and it is directly proportional to the H_2O_2 concentration at the working electrode. Therefore, it also directly proportional to the glucose concentration in solution, thus enabling continuous measurement of saliva glucose.

Evaluation of CA Membrane for Contamination Control. In the MG sensor, AA and UA in the saliva were electrochemically contaminated components, that affected glucose measurement. Since these contaminants were ionized in the solution and negatively charged, CA (Wako) was coated on the electrode as an interference rejection membrane. The suppression performance of contaminants was investigated. The CA was expected to suppress impurities due to the electrostatic repulsion and size effect of the acetate group. In the experiment, a sheet-type electrode applied with an applied constant potential of +400 mV (vs Ag/AgCl) was immersed in phosphate buffer (PB, pH 7.4, 20 mmol/L) to the Pt electrode coated with each interference rejection membrane. The sensor output was compared by loading of each solution of AA (10 μmol/L), UA (100 μmol/L) and H_2O_2 (10 μmol/L). Artificial saliva was prepared from disodium hydrogen phosphate, anhydrous calcium chloride, potassium chloride, sodium chloride, and urea (Kanto Chemical Co., Inc.) according to a protocol reported by Fusayama et al.[41] pH 7.4 PBS was prepared from disodium hydrogen phosphate and potassium dihydrogen phosphate (Kanto Chemical Co.). Glucose solutions were prepared from D(+)-glucose (Wako Pure Chemical Industries Ltd.). In addition, the selectivity of the developed sensor was investigated using the sugar components of galactose, mannitol, fructose, sorbitol, and xylitol as sugar groups.

Evaluation of the MG Glucose Sensor. Measurement of glucose in sampling of human saliva with the glucose biosensor was demonstrated (Figure 2). Saliva was collected from the

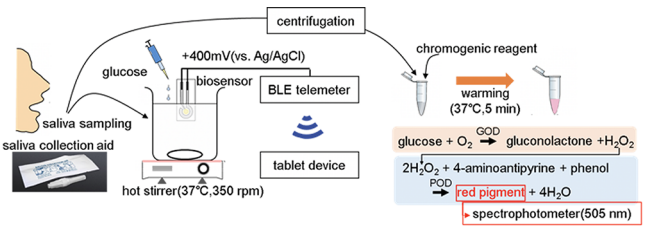


Figure 2. Schematic of the experimental system for measuring glucose concentration in saliva samples

subjects and glucose was measured with a glucose sensor. In saliva collection, saliva was collected using Saliva Collection Aid (Salimetrics, LLC) from a subject who had a meal 2 h or more prior, and oral washing was performed 30 min before collection. In the experiment, the sensor sensitive part was immersed in a beaker filled with the collected saliva, and glucose measurement was performed. Thereafter, a glucose solution was further added, and an output change accompanying an increase in concentration was measured. In order to imitate the measurement state in the oral cavity, a small amount of sample was loaded on the sensitive part, and the

https://dx.doi.org/10.1021/acs.analchem.0c01201
Anal. Chem. XXXX, XXX, XXX−XXX

measurement was performed. In the experiment, (1) saliva sample, (2) saliva sample with increased glucose concentration, and (3) artificial saliva were added to the electrode sensitive parts, and the outputs were compared. For comparison, the salivary glucose concentration was measured using an enzymatic method reagent for glucose measurement (glucose CII test Wako, Wako). This experiment was authorized by the Human Investigations Committee of Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University, (Authorization code: 2015-05), acting up to the Declaration of Helsinki.

Finally, the produced MG sensor was mounted in the oral cavity, and changes in the sensor output were observed. In the experiment, the subject must have undergone a metal allergy patch test in advance (patch test reagent: silver bromide 2% and chloroplatinic acid 0.5%; Torii Pharmaceutical Co. Lld.). The MG-type sensor was installed in the oral cavity of a subject under fasting conditions, and continuous measurement was performed wirelessly to measure the change in output.

RESULTS AND DISCUSSION

Evaluation of the Interference Rejection Membrane. In order to suppress the influence of contamination components of UA and AA in the saliva, the polymeric material of CA of the contamination suppression membrane was coated on the electrode sensitive part. The suppression effect of CA membrane was compared, and the concentration of CA membrane was optimized. The noise ratio (NR) was defined as follows;

$$NR = (\Delta I_{H_2O_2} + \Delta I_{AA} + \Delta I_{UA})/\Delta I_{H_2O_2} \times 100 \quad (1a)$$

In CA (5 wt %), the output of H_2O_2 was 53.0 nA, while the NR was 102%, indicating the possibility of the suppression of contamination (Figure 3). The inhibitory effect was confirmed, and conditions such as the concentration at the time of coating were further investigated for the extent of biological safety of CA. As a result of change in concentration of the CA solution, the NR improved with increasing concentration and saturated at 6 wt %; however, the output of H_2O_2 also decreased. The CA membrane increased the molecular sieving effect by reducing the pore size. Further, the membrane thickness increased, and the transmittance of H_2O_2 decreased. In the subsequent experiments, 5 wt % CA solution was used as an interference rejection membrane as judged by the detection sensitivity of H_2O_2 and NR (97.1 ± 2.0%).

The characteristics of the sensor were investigated using artificial saliva. As shown in Supporting Information (SI) Figure 1, the output decreased as compared with PB, and a stable output current was obtained at each glucose concentration. When quantitative characteristics based on stable values of measurement were examined, the MG sensor in artificial saliva could be quantified in the range of 1.75−10 000 μmol/L, which included the glucose concentration in saliva. The reproducibility of this sensor in phosphate buffer and artificial saliva was evaluated and found to have a high correlation coefficient (R = 0.999). Additionally, the selectivity of the glucose sensor covered with CA membrane was evaluated by comparing the output current in response to 100 μmol/L glucose, galactose, fructose, mannitol, sorbitol and xylitol solutions after 180 s (SI Figure 2). The glucose sensor was shown to be highly selective for glucose based on the substrate specificity of the enzyme of GOD. The device can be

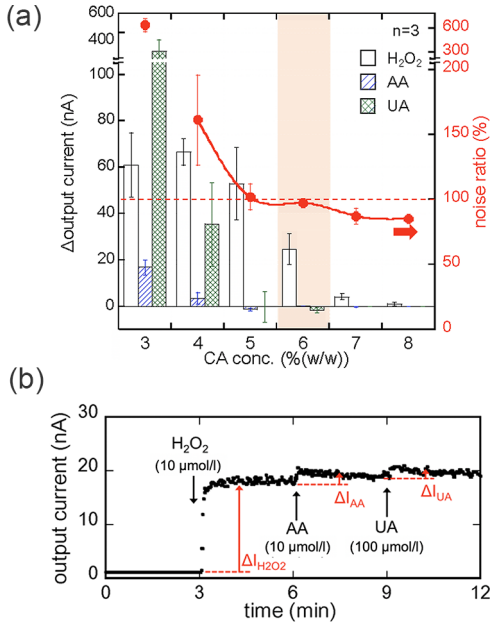


Figure 3. Optimization of CA concentration on the electrode used as an interference rejection membrane. (a) Comparison of the output of each component and noise ratio against CA concentration (hydrogen peroxide, ascorbic acid (AA), and uric acid (UA)). (b) Changes in CA (5%, w/w) coating electrode changes over time when each component was loaded.

washed with a commercially available MG cleaning solution (sodium hypochlorite solution). We confirmed that the output was stable until four times of cleaning.

MG Biosensor Integrated with a Wireless Module. The MG biosensor equipped with a wireless communication module was constructed. With use of a sealing and waterproofing method using a PDMS sheet as the mounting method, we did not observe water ingress into the MG. Sufficient waterproofness was confirmed by this method. The adhesion strength of the PETG material by PDMS was evaluated (Figure 4). The shear strength was 275 mN/mm^2, and the T-type peeling was 47.1 mN/mm^2. The adhesion with PDMS was only in the vicinity of the electrode from the cheek side to the gingival part. The other parts were firmly adhered by thermal welding using a heating gun (T-type peeling: 300

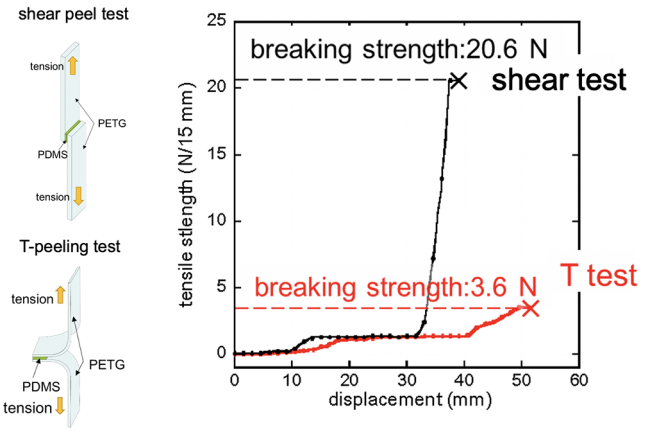


Figure 4. Schematic of the bonding strength tests: shear peel test and T-peeling test. A comparison of bond strength of PETG MG materials by PDMS is shown.

https://dx.doi.org/10.1021/acs.analchem.0c01201
Anal. Chem. XXXX, XXX, XXX−XXX

mN/mm^2). Figure 5 shows the fabricated MG biosensor produced by the above method. The electrode was covered

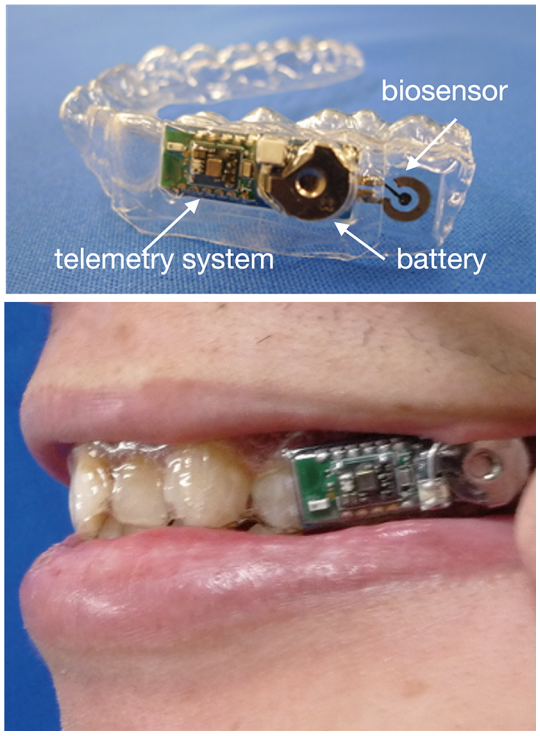


Figure 5. (a) Photograph of the fabricated MG sensor integrated with a wireless module and battery. (b) Wearing of the mouthguard sensor.

with a PDMS sheet, adhered, and hermetically sealed by heat welding, and so it was possible to produce the electrode without damaging it. It was possible to connect the wireless module and the electrodes using a safe and reusable method with the conductive spring.

Artificial saliva glucose containing glucose was introduced to the BLE wireless MG sensor sensitive part. We were able to measure the glucose concentration wirelessly on a mobile terminal with an installed application created in-house. In addition, no water leakage occurred, and it was possible to drive a stable wireless measurement circuit. When the MG sensor was installed in the human oral cavity, it was not apparent in the oral cavity, and the wearability and aesthetic characteristics were excellent as shown in Figure 5.

In Vitro Measurement of Salivary Glucose Using the MG Sensor. The collected saliva sample liquid was directly loaded on the biosensor with CA interference rejection membrane, and the sensor output was measured. The sensor output increased and showed a stable value in the glucose measurements (Figure 6a). The concentration of this output value was calculated from the calibration curve obtained for artificial saliva (Figure 6b). The glucose concentration was estimated to be 5.34 μmol/L. The glucose concentration in the commercial glucose-measurement kit was 27.9 μmol/L, the average concentration for healthy people. When the glucose solution was further loaded during the sensor measurement of the saliva sample, an increase in output depending on the concentration was observed; however, the output at the saliva equivalent glucose concentration (20−200 μmol/L) was higher than that for the artificial saliva. The output decreased by about 70−80% (Figure 6b). In order to investigate the

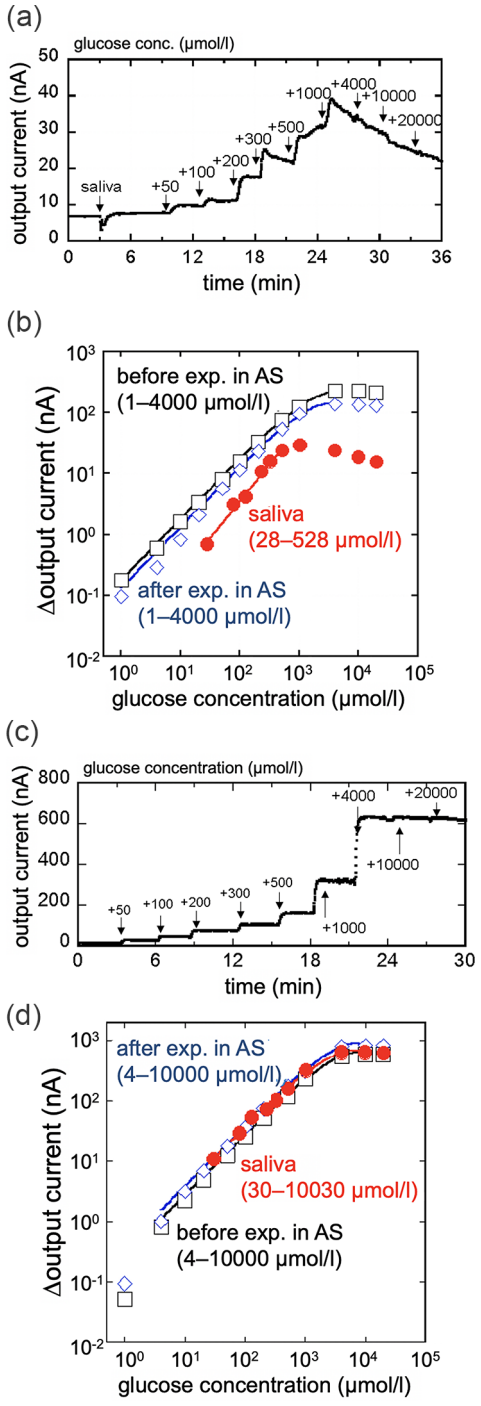


Figure 6. Measurement of salivary glucose using the glucose sensor, (a) response characteristics when untreated sample saliva was loaded with glucose, (b) quantitative characteristics (n = 5), (c) characteristics of glucose response in heat-treated sample saliva, and (d) quantitative characteristics (n = 5) (artificial saliva, AS; phosphate buffer, PB).

cause of the output decrease, the sample saliva heated at 130 °C for 15 min was measured with a biosensor for the reduction of protein and enzyme activities in saliva by heat treatment. The output current in saliva showed good response with the quantitative characteristics of PB. There was no decrease in measurement output for the glucose in saliva. Next, in order to imitate the measurement conditions in the oral cavity, a small amount of sample was loaded on the sensitive part for

https://dx.doi.org/10.1021/acs.analchem.0c01201
Anal. Chem. XXXX, XXX, XXX−XXX

measurement. As a result of applying (1) saliva sample and (2) prepared saliva samples to the biosensor surface, concentration-dependent output was obtained at 200 μmol/L or more, which corresponds to glucose concentration in saliva. As described above, salivary glucose can be detected with the MG sensor with CA membrane. The influence of saliva components could not be completely eliminated in the electrochemical measurements. It was found that further countermeasures against foreign matter were necessary.

In Vivo Measurement of Salivary Glucose Using the MG Sensor. The developed MG biosensor was installed in the oral cavity and saliva glucose was measured. The influence of pH on the pH of this sensor was examined for evaluation in the human oral cavity. With adjustment to the temperature of the oral cavity, 38 °C, a stable output was obtained for a range of pH in saliva (SI Figure 3). In the in vivo measurement, the current value was stabilized in pure water, and then it was mounted in the subject's mouth to observe the output signal. As shown in Figure 7, the output increased rapidly to about 30

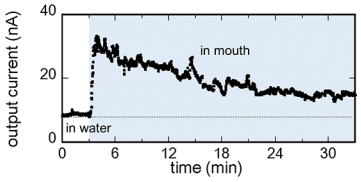


Figure 7. Output current response when MG biosensor was installed and mounted in the human oral cavity. The glucose concentration in human saliva was estimated to be 21.1 μmol/L using the calibration curve.

nA as it was installed in the oral cavity, and then the output decreased gradually over time and showed a stable value in about 20 min. In addition, no significant disturbance in output current due to the MG device was observed. Using the difference between the steady value in deionized water and the stable value after wearing as the sensor output, we estimated the glucose concentration in saliva in the oral cavity to be 21.1 μmol/L from the calibration curve for artificial saliva. The glucose concentration was calculated from the saliva sample collected before wearing. The result for the glucose measurement kit and spectrophotometer showed 17.6 μmol/L. The result of glucose concentration using the MG sensor in the oral cavity was mostly consistent with the experiment result of the glucose measurement kit. As described above, the influence of contaminants has been confirmed in the measurement with the saliva sample. In the future, it will be necessary to improve the sensor and measure the salivary glucose in the oral cavity under various conditions to confirm the effectiveness of the MG sensor. Moreover, this biosensor was shown to be able to measure low concentrations of glucose in saliva. In the future, this sensor has sufficient potential for the evaluation of hypoglycemic conditions.

CONCLUSIONS

In this work, we investigated an interference rejection membrane and improved the selective film technology for the MG glucose sensor for the purpose of noninvasive and direct measurement of glucose in saliva. By coating CA on the electrode used as an interference rejection membrane, it was possible to suppress the influence of contaminants of AA and UA at noise ratio of 97.1%. An application for mobile terminals equipped with Android OS was developed, and a wireless measurement environment with excellent portability was realized. A stable output was obtained with the MG biosensor in the human oral cavity. The possibility of continious salivary glucose measurement was suggested. In the future, it is expected that the sensor will be further improved with respect to the factor of the decrease in saliva measurement sensitivity. Measurement of salivary glucose in the human oral cavity will be realized for noninvasive evaluation of concentration correlated with blood glucose.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.analchem.0c01201.

Figure S1: quantitative characteristics of sheet-type glucose sensor; Figure S2: selectivity of the mouthguard biosensor; Figure S3: the response of the sensor to pH condition (PDF)

AUTHOR INFORMATION

Corresponding Authors

Takahiro Arakawa − *Department of Biomedical Devices and Instrumentation, Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University, Chiyoda-ku, Tokyo 101-0062, Japan*; Phone: +81-3-5280-8093; Email: arakawa.bdi@tmd.ac.jp; Fax: +81-3-5280-8094

Kohji Mitsubayashi − *Department of Biomedical Devices and Instrumentation, Institute of Biomaterials and Bioengineering and Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Chiyoda-ku, Tokyo 101-0062, Japan*; orcid.org/0000-0002-0709-4957; Phone: + 81-3-5280-8091; Email: m.bdi@tmd.ac.jp; Fax: +81-3-5280-8094

Authors

Keisuke Tomoto − *Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Bunkyo-ku, Tokyo 113-8510, Japan*

Hiroki Nitta − *Department of Biomedical Devices and Instrumentation, Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University, Chiyoda-ku, Tokyo 101-0062, Japan*

Koji Toma − *Department of Biomedical Devices and Instrumentation, Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University, Chiyoda-ku, Tokyo 101-0062, Japan*

Shuhei Takeuchi − *Gerodontology and Oral Rehabilitation, Tokyo Medical and Dental University, Bunkyo-ku, Tokyo 113-8549, Japan*

Toshiaki Sekita − *Gerodontology and Oral Rehabilitation, Tokyo Medical and Dental University, Bunkyo-ku, Tokyo 113-8549, Japan*

Shunsuke Minakuchi − *Gerodontology and Oral Rehabilitation, Tokyo Medical and Dental University, Bunkyo-ku, Tokyo 113-8549, Japan*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.analchem.0c01201

Notes

The authors declare no competing financial interest.

https://dx.doi.org/10.1021/acs.analchem.0c01201
Anal. Chem. XXXX, XXX, XXX−XXX

■ ACKNOWLEDGMENTS

This work was partly supported by Japan IDDM network, Department of Citizens and Environmental Affairs and Japan Society for the Promotion of Science (JSPS) KAKENHI 16KK0143 and 19H02150 by the Ministry of Education, Culture, Sports, Science and Technology (MEXT). We thank Uniedit for editing and proofreading this manuscript.

■ REFERENCES

(1) Itabashi, K.; Yamada, Y.; Hiyama, S.; Tabata, H.; Toyooka, T.; Takeuchi, S.; Okubo, T.; Itabashi, K.; Okubo, T.; Tabata, H. *Anal. Chem.* **2015**, *87* (15), 7588−7594.
(2) Kaushik, A.; Vasudev, A.; Arya, S. K.; Pasha, S. K.; Bhansali, S. *Biosens. Bioelectron.* **2014**, *53*, 499−512.
(3) Nam, H. C.. IDF Diabetes Atlas Eighth Edition 2017; **2017**. DOI: 10.1016/S0140-6736(16)31679-8.
(4) Neville, S. E.; Boye, K. S.; Montgomery, W. S.; Iwamoto, K.; Okamura, M.; Hayes, R. P. *Diabetes/Metab. Res. Rev.* **2009**, *25* (8), 705−716.
(5) Shepherd, P. R.; Kahn, B. B. *N. Engl. J. Med.* **1999**, *341* (4), 248−257.
(6) Chen, L.; Magliano, D. J.; Zimmet, P. Z. *Nat. Rev. Endocrinol.* **2012**, *8* (4), 228−236.
(7) Kim, J.; Campbell, A. S.; Wang, J. *Talanta* **2018**, *177* (July 2017), 163−170.
(8) Viswanath, B.; Choi, C. S.; Lee, K.; Kim, S. *TrAC, Trends Anal. Chem.* **2017**, *89*, 60−67.
(9) Sieg, A.; Guy, R. H.; Delgado-Charro, M. B. *J. Pharm. Sci.* **2003**, *92* (11), 2295−2302.
(10) Bandodkar, A. J.; Jia, W.; Yardimci, C.; Wang, X.; Ramirez, J.; Wang, J. *Anal. Chem.* **2015**, *87* (1), 394−398.
(11) Guy, R. *Nat. Nanotechnol.* **2016**, *11* (6), 493−494.
(12) Lee, H.; Song, C.; Hong, Y. S.; Kim, M. S.; Cho, H. R.; Kang, T.; Shin, K.; Choi, S. H.; Hyeon, T.; Kim, D. H. *Sci. Adv.* **2017**, *3* (3), 1−9.
(13) Liu, L.; Zhang, D.; Zhang, Q.; Chen, X.; Xu, G.; Lu, Y.; Liu, Q. *Biosens. Bioelectron.* **2017**, *93* (June2016), 94−101.
(14) Chien, P. J.; Suzuki, T.; Tsujii, M.; Ye, M.; Minami, I.; Toda, K.; Otsuka, H.; Toma, K.; Arakawa, T.; Araki, K.; et al. *Anal. Chem.* **2017**, *89* (22), 12261−12268.
(15) Ye, M.; Chien, P. J.; Toma, K.; Arakawa, T.; Mitsubayashi, K. *Biosens. Bioelectron.* **2015**, *73*, 208−213.
(16) Bailey, T.; Bode, B. W.; Christiansen, M. P.; Klaff, L. J.; Alva, S. *Diabetes Technol. Ther.* **2015**, *17* (11), 787−794.
(17) Miyashita, M.; Ito, N.; Ikeda, S.; Murayama, T.; Oguma, K.; Kimura, J. *Biosens. Bioelectron.* **2009**, *24* (5), 1336−1340.
(18) Corrie, S. R.; Coffey, J. W.; Islam, J.; Markey, K. A.; Kendall, M. A. F. *Analyst* **2015**, *140* (13), 4350−4364.
(19) Zaryanov, N. V.; Nikitina, V. N.; Karpova, E. V.; Karyakina, E. E.; Karyakin, A. A. *Anal. Chem.* **2017**, *89* (21), 11198−11202.
(20) Parrilla, M.; Guinovart, T.; Ferré, J.; Blondeau, P.; Andrade, F. J. *Adv. Healthcare Mater.* **2019**, *8* (16), 1900342.
(21) Karpova, E. V.; Shcherbacheva, E. V.; Galushin, A. A.; Vokhmyanina, D. V.; Karyakina, E. E.; Karyakin, A. A. *Anal. Chem.* **2019**, *91* (6), 3778−3783.
(22) Kim, J.; Campbell, A. S.; De Ávila, B. E.; Wang, J. *Nat. Biotechnol.* **2019**, *37*, 389−406.
(23) Bruen, D.; Delaney, C.; Florea, L.; Diamond, D. *Sensors* **2017**, *17* (8), 1−21.
(24) Bandodkar, A. J.; Wang, J. *Trends Biotechnol.* **2014**, *32* (7), 363−371.
(25) Jia, W.; Bandodkar, A. J.; Valdés-Ramírez, G.; Windmiller, J. R.; Yang, Z.; Ramírez, J.; Chan, G.; Wang, J. *Anal. Chem.* **2013**, *85* (14), 6553−6560.
(26) Sempionatto, J. R.; Nakagawa, T.; Pavinatto, A.; Mensah, S. T.; Imani, S.; Mercier, P.; Wang, J. *Lab Chip* **2017**, *17* (10), 1834−1842.
(27) Chu, M. X.; Shirai, T.; Takahashi, D.; Arakawa, T.; Kudo, H.; Sano, K.; Sawada, S. I.; Yano, K.; Iwasaki, Y.; Akiyoshi, K.; et al. *Biomed. Microdevices* **2011**, *13* (4), 603−611.
(28) Mitsubayashi, K.; Arakawa, T. *Electroanalysis* **2016**, *28*, 1170−1187.
(29) Tiongco, R.; Bituin, A.; Arceo, E.; Rivera, N.; Singian, E. *J. Clin. Exp. Dent.* **2018**, *10* (9), 0−0.
(30) Du, Y.; Zhang, W.; Wang, M. L. *J. Diabetes Sci. Technol.* **2016**, *10* (6), 1344−1352.
(31) Kim, J.; Imani, S.; de Araujo, W. R.; Warchall, J.; Valdés-Ramírez, G.; Paixão, T. R. L. C.; Mercier, P. P.; Wang, J. *Biosens. Bioelectron.* **2015**, *74*, 1061−1068.
(32) Arakawa, T.; Kuroki, Y.; Nitta, H.; Chouhan, P.; Toma, K.; Sawada, S.; Takeuchi, S.; Sekita, T.; Akiyoshi, K.; Minakuchi, S.; et al. *Biosens. Bioelectron.* **2016**, *84*, 106−111.
(33) Valdés-Ramírez, G.; Bandodkar, A. J.; Jia, W.; Martinez, A. G.; Julian, R.; Mercier, P.; Wang, J. *Analyst* **2014**, *139* (7), 1632−1636.
(34) García-Carmona, L.; Martín, A.; Sempionatto, J. R.; Moreto, J. R.; González, M. C.; Wang, J.; Escarpa, A. *Anal. Chem.* **2019**, *91* (21), 13883−13891.
(35) de Castro, L. F.; de Freitas, S. V.; Duarte, L. C.; de Souza, J. A. C.; Paixão, T. R. L. C.; Coltro, W. K. T. *Anal. Bioanal. Chem.* **2019**, *411* (19), 4919−4928.
(36) Ilea, A.; Andrei, V.; Feurdean, C.; Băbţan, A.-M.; Petrescu, N.; Câmpian, R.; Boşca, A.; Ciui, B.; Tertiş, M.; Săndulescu, R.; et al. *Biosensors* **2019**, *9* (1), 27.
(37) Wataha, J. C. *J. Prosthet. Dent.* **2000**, *83* (2), 223−234.
(38) Park, G.; Chung, H. J.; Kim, K.; Lim, S. A.; Kim, J.; Kim, Y. S.; Liu, Y.; Yeo, W. H.; Kim, R. H.; Kim, S. S.; et al. *Adv. Healthcare Mater.* **2014**, *3* (4), 515−525.
(39) Iwasaki, Y.; Ishihara, K. Cell Membrane-Inspired Phospholipid Polymers for Developing Medical Devices with Excellent Biointerfaces. *Sci. Technol. Adv. Mater.* **2012**, *13* (6). 064101.
(40) Toma, K.; Tomoto, K.; Yokota, K.; Yasuda, N.; Ishikawa, T.; Arakawa, T.; Mitsubayashi, K. *Sens. Mater.* **2018**, *30* (12), 3053.
(41) Fusayama, T.; Katayori, T.; Nomoto, S. *J. Dent. Res.* **1963**, *42* (5), 1183−1197.

https://dx.doi.org/10.1021/acs.analchem.0c01201
Anal. Chem. XXXX, XXX, XXX−XXX