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Reactant/polymer hybrid films on p-n junction photodetectors for self-powered, non-invasive glucose biosensors

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ABSTRACT

The portability of electronic-based biosensors is limited because of the use of batteries and/or solutions containing reactants such as enzymes for assay, which limits the utility of such biosensors in point-of-care (POC) testing. In this study, we report on the development of a self-powered biosensor composed of only portable components: a reactant-containing poly (ethylene glycol) (PEG) film for the colorimetric assay, and a self-powered n-InGaZnO/p-Si photodetector. The PEG film containing enzymes and color-developing agents was formed on a glass slide by spin coating. The self-powered biosensor was fabricated by placing the hybrid film on the p-n junction photodetector, and applied in non-invasive glucose detection (salivary glucose). Injection of the target-containing solution dissolved the PEG that led to the release of enzymes and color-developing agents, resulting in a colorimetric assay. The colorimetric assay could attenuate the light reaching the photodetector, thus facilitating target concentration verification by measuring the photocurrent. Our self-powered biosensor has two main advantages: (i) all components of the biosensor are portable and (ii) dilution of target concentration is avoided as the reagents are in the PEG film. Therefore, the self-powered biosensor, without solution-phase components, could be highly beneficial for creating portable, sensitive biosensors for POC testing.

1. Introduction

Healthcare trends have been shifting from hospital-centered to individual-centered testing by using personalized biosensors that enable self-testing, also called point-of-care (POC) testing, which does not require laboratory staff or equipment (Luppa et al., 2011; Gervais et al., 2011). Among various types of biosensor platforms, electronic (typically, electrochemical or piezoresistive) devices have been attracted much attention for POC biosensor platforms due to the high sensitivity, ability to perform quantitative analyses, ease of miniaturization, and potential integration with smart devices (Yu et al., 2019a, 2019b, 2020; Liu et al., 2020). An increasing need exists to develop multiple electronic biosensor arrays for self-diagnosis of various diseases simultaneously and accuracy enhancement by conducting statistical analyses (Albert et al., 2000; Gao et al., 2016). In this regard, one of obstacles to develop such self-testing devices is the use of portable power supplies such as a battery for those operations owing to suffering from recharging, life time, and insufficient capacity issues as well as complexity of sensor components (Wang, 2010, 2012; Lv et al., 2018). Hence, battery-free operation of electronic biosensors is highly attractive in terms of portability, use with convenience, simplicity of the biosensor components.

A self-powered biosensor platform, which can be operated via energy derived from physical pressure, spontaneous chemical reactions, or light, is an innovative approach to solve existing power supply limitations (Wang, 2010, 2012; Lv et al., 2018; Kim et al., 2018; Yu et al., 2018). Biofuel cells (electrochemical), piezoelectric, and thermoelectric devices have been extensively studied for application as self-powered biosensors (Wang, 2012; Grattieri and Minteer, 2018; Huang et al., 2020). In such studies, bioreceptors such as enzymes, antibodies, and DNA were directly immobilized onto electrodes (or active materials) to detect targets. Such sensor systems suffered from lower sensitivity and stability towards the targets in real samples (such as saliva and urine), owing to the matrix effect stemming from direct contact between the electrodes and electroactive species in the real sample (Yogeswaran and Chen, 2008; Grattieri and Minteer, 2018; Zhang and Chen, 2019). In

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recent times, our group reported on a self-powered biosensor that exhibited no matrix effect by performing an enzyme-based colorimetric assay on a p-n junction photodetector (solar cell) (Kim et al., 2018). The colorimetric reaction attenuated the light reaching the p-n junction photodetector, and thus, a decrease in the photocurrent was used as a quantitative target analysis. The self-powered biosensor exhibited high and stable sensitivity towards the targets in body fluids due to the separation between the solution for the colorimetric assay (reaction part) and the photodetector (detector part).

Nonetheless, the self-powered biosensor was not portable enough for individuals because a solution, containing the enzymes and color-developing agents, was used for the colorimetric assay–the solution is inconvenient for storage and transport. Moreover, target injection to the reagent-containing solution resulted in dilution of the target concentration, making the biosensor difficult to detect the target at low concentration. Therefore, self-powered biosensor platforms featuring p-n junction devices, and specifically non-solution-phase components, are necessary to develop highly sensitive, portable POC biosensors.

In this study, we reported on a self-powered biosensor—composed of all-portable matter—realized by placing a reagent-containing poly (ethylene glycol) (PEG) film on a self-powered n-InGaZnO (IGZO)/p-Si photodetector, which showed a high and stable sensitivity towards the target in the non-diluted (100%) real human sample. PEG is a highly water-soluble polymer (Pietrelli, 2013), and thus, the reactants—enzymes and color-developing elements for the colorimetric assay—could be released from the PEG film when exposed to the target solution. As a model study, our self-powered biosensor was applied in non-invasive glucose detection (salivary glucose) for diabetic care; diabetes is worldwide chronic disease that threatens human health and lives (Li et al., 2021). Especially, non-invasive glucose detection is in high demand due to the process being painless, having no risk of infection and damage caused to the tissue by finger-pricking, and the possibility of frequent measurements (Villena et al., 2019; Lin et al., 2019). Despite the low glucose concentrations in saliva (approximately 100 times lower as compared to that in blood) (Macaya et al., 2007), our self-powered biosensor could successfully trace the salivary glucose levels.

2. Experimental section

2.1. Materials

D-(+)-glucose (BioXtra, ≥ 99.5), poly (ethylene glycol) (PEG, M_n = 400), and 4-aminoantipyrine (4-AAP, reagent grade) were obtained from Sigma Aldrich. Glucose oxidase (GOx, GLO-201, 100 U mg^{-1}) and horseradish peroxidase (HRP, PEO-301, 110 U mg^{-1}) were purchased from Toyobo Co., Ltd. (OSK, Japan). N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methoxyaniline sodium salt dihydrate (ADOS) was purchased from Dojindo Molecular Technologies, Inc. (Tokyo, Japan). Polydimethylsiloxane (PDMS, SYLGARD 184) was purchased from Dow Corning (USA). Phosphate buffered saline (PBS, pH 7.4) was purchased from Biosesang (Seongnam, South Korea). The white light emitting diode (LED, PP00W-8L61-Star 1 W WHITE Power LED) was purchased from Photron (Anseong, Korea). The absorbance spectra were measured using a Cytation 5 instrument (BioTek Instruments, Inc., USA).

2.2. Fabrication of p-n photodetectors

A p-n junction photodetector was fabricated as reported in our previous work (Kim et al., 2018). In brief, a p-n junction was formed by depositing an n-type InGaZnO (IGZO) thin film (50 nm thickness) on a p-type silicon wafer by DC magnetron sputtering. Silver was deposited to form the top electrode using an e-beam evaporator on the IGZO/Si substrate, which was covered by an antennae shape shadow mask, and a copper tape was attached as the bottom electrode onto the back-side of silicon wafer wherein IGZO was not deposited.

2.3. Fabrication of reactants/PEG hybrid film on a glass slide

HRP (5000 U mL^{-1}), GOx (5000 U mL^{-1}) 4-AAP (0.5 M), and ADOS (0.5 M) were mixed with PEG in the volume ratio of 1:1:1:1:0.5. The well-mixed solution was spin coated on a glass slide (10 mm (length) × 15 mm (width) × 0.7 mm (height)) at 2000 rpm for 60 s.

2.4. Fabrication of PDMS vessel to place the hybrid film-coated glass slide

PDMS and the curing agent were mixed in the ratio of 10:1 and stirred for 30 min. The mixture was poured on a glass slide (10 mm (length) × 15 mm (width) × 1.4 mm (height)) that was attached to a Petri dish. The bubbles in the PDMS were eliminated using a vacuum pump, and PDMS was cured in an oven at 65 °C for 3 h. A PDMS vessel was obtained by cutting and detaching the cured PDMS from the Petri dish and the glass slide (10 mm (length) × 15 mm (width) × 1.4 mm (height)).

2.5. Developing the self-powered biosensor

The glass slide coated with the reactant/PEG hybrid film was placed in the PDMS vessel. To place the PDMS on the IGZO/Si junction photodetector, cases for each part were fabricated using a 3D printer (Ultimaker 3) as reported in our previous work (Kim et al., 2018). The PDMS vessel placed on the IGZO/Si junction photodetector was the final form of the self-powered biosensor. It should be noted that a glass slide was placed on the PDMS vessel, which partially covered the vessel, but completely covered the pathway for the light to reach the self-powered photodetector. This was important to avoid photocurrent changes originating from the morphology of the injected solution for glucose detection.

2.6. Glucose detection using the self-powered biosensor

Glucose detection was carried out by injecting the glucose-containing solution into a PDMS vessel wherein a reactant-containing PEG film was placed. After 5 min from the injection, a photocurrent was obtained by conducting transient mode measurements using a Compactstat (Ivium technologies, Netherlands), by applying 0 V. A white light emitting diode (LED) was used as the light source.

3. Results and discussion

A self-powered biosensor was developed by placing a reactant/PEG hybrid film in a poly (dimethylsiloxane) (PDMS) vessel and subsequently placing the vessel onto an n-IGZO/p-Si junction photodetector (Fig. 1a). The self-powered biosensor works on the principle of attenuating the light reaching the p-n junction photodetector via an enzyme-based colorimetric assay stemming from the reaction between reactants in the hybrid film and glucose, resulting in a decrease in the photocurrent (Fig. 1b). The color development begins with the oxidation of glucose by glucose oxidase (GOx), resulting in the production of hydrogen peroxide (H_2O_2). Subsequently, horseradish peroxidase (HRP), in the co-presence of H_2O_2 and 4-aminoantipyrine (4-AAP), oxidizes the colorless N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methoxyaniline (ADOS) to its purple oxidized form (Fig. 1b). Similar to solar cells, a p-n junction photodetector can produce photocurrent without the application of any bias on the photodetector due to the p-n junction. For the hybrid film, PEG—a common water-soluble polymer used for chemical, biological, and medical uses—was chosen to contain the components for the colorimetric assay. To fabricate the hybrid film, the components (HRP, GOx, ADOS, and 4-AAP) were mixed with PEG and spin coated onto a glass side (Fig. 1c). The function of the PEG film was to contain the reactants for the colorimetric assay, and to release the same when the PEG was exposed to the target solution due to the highly water-soluble nature of PEG.

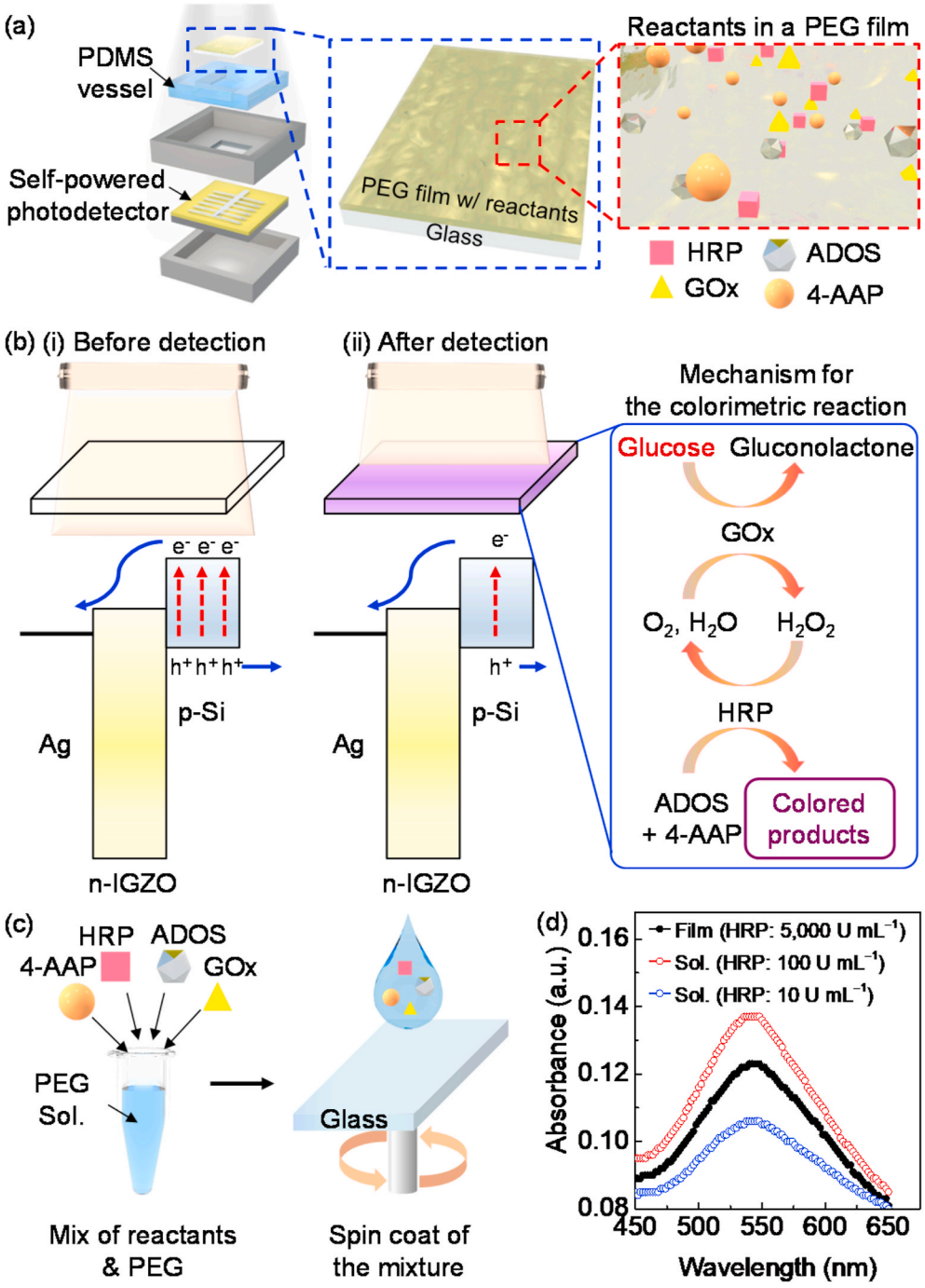

Fig. 1. Schematics of (a) the self-powered biosensor composed of portable components. (b) The working principle of the self-powered biosensor. (c) Fabrication method of the reactant-containing polymer film for the colorimetric assay. (d) Absorbance spectra of the reactant-containing polymer film (black), 100 U mL^{-1} HRP solution (red), and 10 U mL^{-1} HRP solution (blue). The reactants were composed of HRP, GOx, ADOS and 4-AAP; glucose concentration for the colorimetric reaction was 1.0 mg dL^{-1}. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

The glucose-detection ability of the hybrid film on the glass slide was evaluated by dropping 1 mg dL^{-1} glucose (100 μL) onto the film. Five minutes after dropping the glucose solution, the absorption spectrum of the hybrid film on the glass was measured (Fig. 1d). A significantly larger amount of HRP was required to achieve a similar color in comparison to that of the combination of HRP, GOx, ADOS, and 4-AAP together in solution.

The hybrid film on the glass was further evaluated via exposure to various concentrations of glucose (Fig. S1). An increase in the color-developing phenomenon was observed with an increase in glucose concentration in the range of 0.6–2.0 mg dL^{-1} (Fig. S1a). The absorbances were measurable with a resolution of 0.2 mg dL^{-1} (Fig. S1b).

A digital image of the photodetector is shown in Fig. 2a. A white LED light source was used to generate the photocurrent in the biosensor, and its spectrum overlapped with that of the oxidized ADOS (Fig. 2b). Although the white LED was chosen to operate the self-powered biosensor in this work, various visible light sources observed in daily life environment, such as fluorescent light and sunlight, could also be also used for the operation of the biosensor (in the case of sunlight–varying intensities at each moment, dual self-powered biosensors had to be used for current calibration), as reported in our previous work (Kim et al., 2018). This is because Si can absorb the entire visible light wavelength due to its band gap of 1.1 eV. The photocurrent of the self-powered biosensors decreased with an increase in the glucose concentration in deionized water (D.W.) with a resolution of 0.2 mg dL^{-1}, within the range of 0.8–1.8 mg dL^{-1} (Fig. 2c).

Furthermore, glucose spiked in the pooled human saliva was also detected using the self-powered biosensors. The glucose concentration

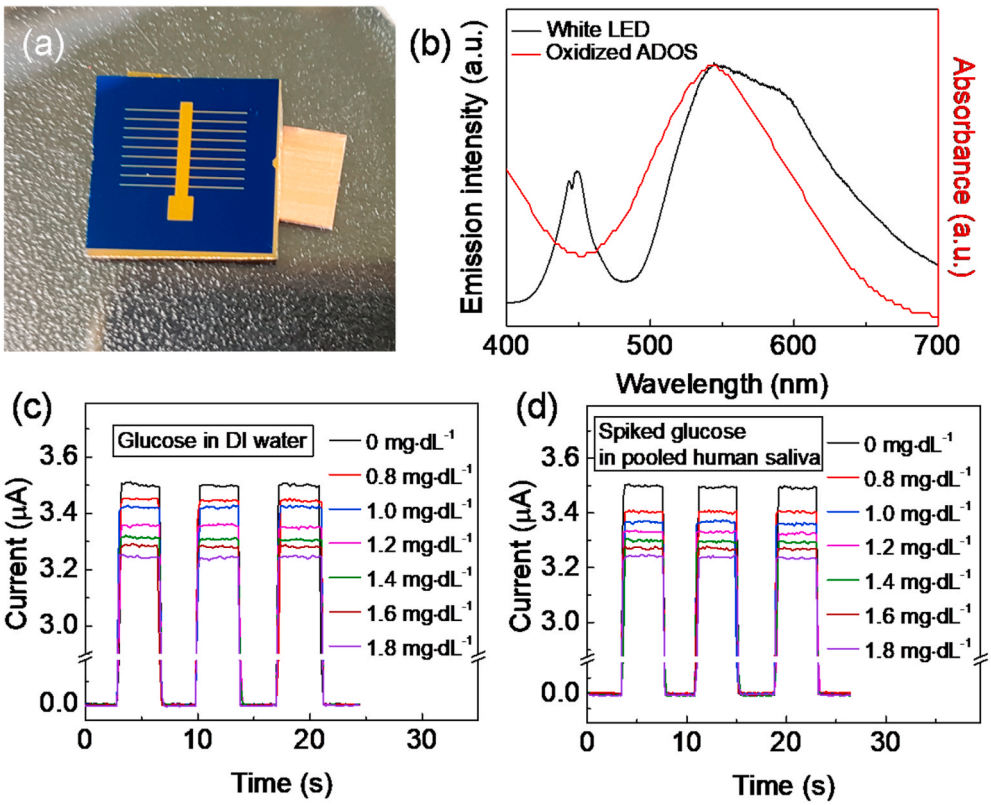

Fig. 2. (a) Digital image of the IGZO/Si junction photodetector. (b) Emission and absorbance spectra of the white LED and ADOS, respectively. (c) and (d) show the photocurrents measured using the self-powered biosensor at different glucose concentrations in D.W. and pooled human saliva, respectively; a light source was the white LED.

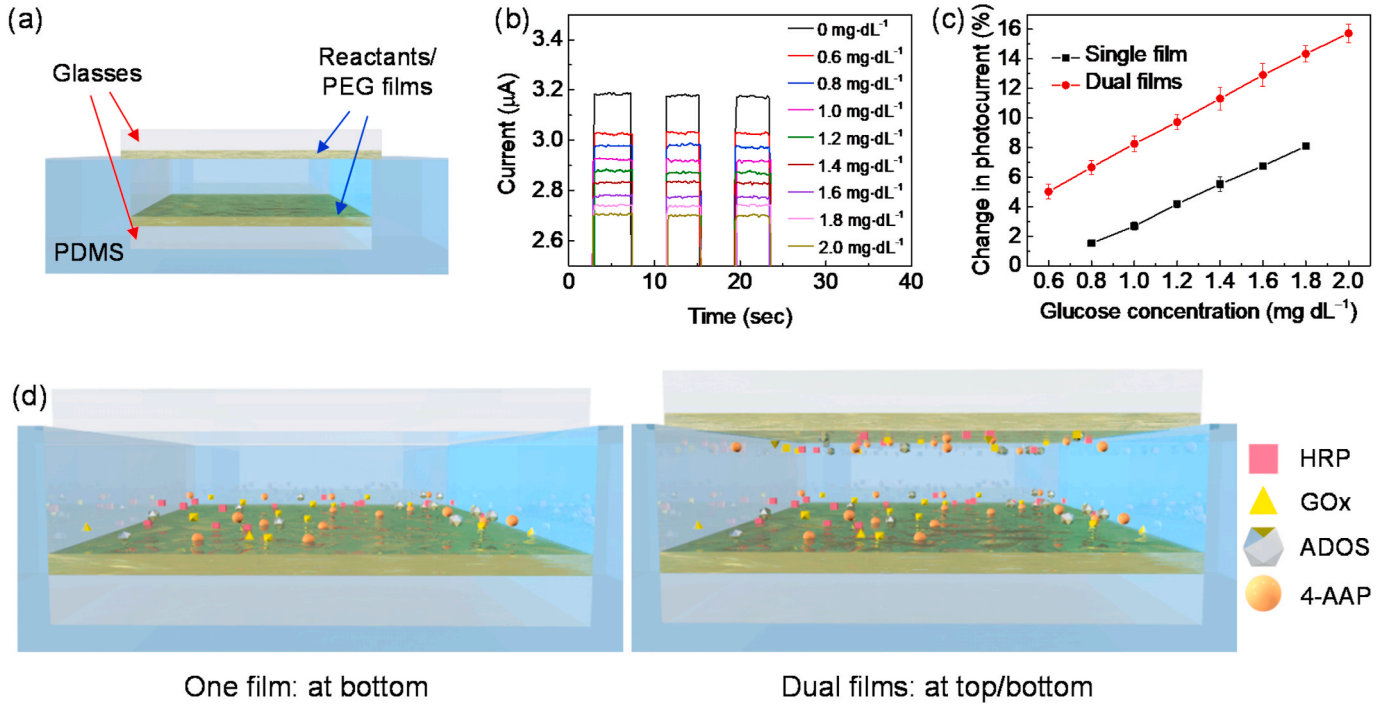

Fig. 3. (a) Schematic of the dual reactant-containing PEG films in PDMS vessel. (b) Glucose detection using a self-powered biosensor composed of the dual films and the IGZO/Si photodetector. The figure displays the photocurrent measured at different glucose concentrations. (c) Change in photocurrent measured at different glucose concentrations. A linear change in the photocurrent with variations in glucose concentration is observed: single film (black) and dual film (red). (d) Schematic illustrations for release of reactants from single film (left) and dual films: at the top and bottom (right). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

in the purchased pooled human saliva was 0.8 mg dL^{-1}, which was analyzed using a clinical analyzer (Hitachi 7020 Clinical Analyzer; Hitachi High-Technologies Corporation, Japan). The glucose spiked in saliva was successfully detected within the range of 0.8–1.8 mg dL^{-1} and with a resolution of 0.2 mg dL^{-1} (Fig. 2d). It was noteworthy that although 100% salivary glucose was used for the detection without dilution, no matrix effect was observed as compared to the glucose detection in D.W. This was because the detection and reaction compartments (a self-powered photodetector and a solution for the assay, respectively) were physically separated, thereby avoiding direct contact between the 100% saliva and the active materials at the self-powered photodetector. Additionally, no undesired colorimetric reaction was caused by the chemically activated species in the saliva, which was confirmed by comparing the photocurrents while injecting the 100% saliva and 0.8 mg dL^{-1} glucose in the buffer solution (Fig. S2).

To enhance the sensitivity of the self-powered biosensors, two reactant-containing hybrid films were placed in a PDMS vessel as shown in Fig. 3a. The self-powered biosensor with the dual hybrid films exhibited an increased sensitivity towards glucose within the range of 0.6–2.0 mg dL^{-1}, with a resolution of 0.2 mg dL^{-1} (Fig. 3b). Percentage changes in the photocurrents using the dual films, within 0.2 mg dL^{-1} of glucose, were higher than those of the single film: 0.7% and 1.8% while using the single and dual films, respectively (Fig. 3c). The graphs for the single and dual films were obtained after repeating the experiment five times. As a result, the R^2 values for the single and dual films were 0.999 each. The sensitivity was enhanced by over two times. This was not only because of doubling the amount of reactants but also due to the reactants being able to diffuse from both the top and bottom sides instead of a single side (Fig. 3d). Considering that salivary glucose levels lie in the range of 0.144–3.78 mg dL^{-1} (Macaya et al., 2007), the glucose detection range of 0.6–2.0 mg dL^{-1} and resolution of 0.2 mg dL^{-1} (Figs. 2d and 3b) are satisfactory results for the potential application of self-powered biosensors in non-invasive glucose detection.

Stabilities of the reactant-containing polymer films and reactants in the solution were evaluated (Fig. S3). During the stability testing period, the films and solution were stored at 4 °C and protected from light. Glucose detection abilities of the reactant-containing films and solution were evaluated daily by dropping and injecting 40 μL and 5 μL of glucose solutions (200 mg dL^{-1}) onto the films and reactant solution (45 μL), respectively. After 1 min, the absorbance intensities were measured. The reactants in the film showed more stable sensitivity towards glucose than those in the solution during 2 weeks, indicating that the components were stably stored in PEG.

Therefore, the advantages of employing reactant-containing hybrid films in self-powered systems are as follows: (i) all the components were in the solid phase, which made the electronic biosensor portable (the solution is not a portable matter; thus, it is undesirable for POC biosensors) (Guan et al., 2015; Han et al., 2017); and (ii) dilution of the target solution did not occur while the target (glucose) solution was injected because all the components for the colorimetric assay were in the solid phase, which led to a higher sensitivity and resolution as compared to those afforded by the solution phase component.

4. Conclusions

In this study, we successfully demonstrated a self-powered biosensor system that consisted of all solid-phase components: a reactant/PEG hybrid film was placed above an n-IGZO/p-Si photodetector. The hybrid film was obtained by blending all the components required for the colorimetric assay—HRP, GOx, ADOS, and 4-AAP—with PEG, and subsequently spin coating the mixture solution on a glass slide. The self-powered biosensor could detect glucose in 100% pooled human saliva without dilution within a range of 0.6–2.0 mg dL^{-1}, with a resolution of 0.2 mg dL^{-1}. Such levels of glucose detection in 100% saliva was possible due to the physical separation of the p-n junction photodetector and the saliva. Additionally, the sensitivity was amplified using the dual hybrid films. In this self-powered system, all the components were in the solid phase, thereby making the system portable. Furthermore, any undesired dilution of the target (glucose) solution was avoided, which resulted in high sensitivity and resolution for the colorimetric assay. Based on such merits, our self-powered biosensor successfully traced salivary glucose levels with a simple injection of 100% saliva, which was highly advantageous for non-invasive, on-site glucose measurements. Although the present model of the self-powered biosensors needs a current meter, it can be expected that this self-powered platform can be integrated with Bluetooth, Wi-Fi, or radio frequency identification modules to interconnect with smart devices to measure photocurrent in the future work. Therefore, our self-powered biosensors could be considered as promising candidates for future electronic POC applications.

CRediT authorship contribution statement

Kihyeun Kim: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Visualization, Writing - original draft, Writing - review & editing. **Hyeonghun Kim:** Methodology, Validation, Formal analysis, Investigation. **Eun-Jung Jo:** Methodology, Formal analysis, Investigation, Writing - original draft. **Hyungjun Jang:** Methodology, Investigation. **Jiyoon Park:** Methodology, Investigation. **Gun Young Jung:** Investigation, Writing - review & editing. **Min-Gon Kim:** Conceptualization, Writing - review & editing, Funding acquisition, Resources, Supervision, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bios.2020.112855.

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