PARYLENE BASED FLEXIBLE GLUCOSE SENSOR USING GLUCOSE-RESPONSIVE FLUORESCENT HYDROGEL

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ABSTRACT

We propose a flexible glucose sensor combined with a parylene based electrode and a glucose-responsive fluorescent hydrogel for continuous monitoring of glucose concentration. By depositing parylene-C on a cupper (Cu) thin sheet and creating electrical circuits including a LED and a photo diode, we developed the flexible glucose sensor. The flexible glucose sensor could reduce damage to tissues and organs of patients because the flexibility allows its deformation following movements of the patients. Using the flexible glucose sensor, we succeeded in wireless measurement of glucose concentration in vivo by implantation of the sensor into a rat.

INTRODUCTION

Continuous glucose monitoring is the key to improve the quality of lives of patients with diabetes mellitus compared with conventional glucose measuring method using puncture needle because the continuous glucose monitoring allows detect sudden glucose level change and prevent hyperglycemia [1]. Measurement of in vivo blood glucose level using implantable devices is one of the promising way to enable the continuous glucose monitoring. Glucose-responsive hydrogel is a desirable material for owing to its high durability compared with conventional enzymatic electrodes and its flexibility [2-6]. These properties allow long-term glucose monitoring and reduction of physical damage to patient's tissue. The glucose-responsive hydrogel generates fluorescence according to glucose concentration. Previous research reported that implantable glucose-responsive hydrogel achieved continuous glucose monitoring transdermally by exposure of excitation lights through the skin [7, 8]. However, the transdermal measurement using the fluorescence of the glucose-responsive hydrogel and a photo detector is vulnerable to exogenous noise because the position of the detector can change as a patient move. Therefore, developing an implantable flexible glucose sensor that can excite the glucose-responsive hydrogel and detect the generated fluorescence of the hydrogel subcutaneously is important to monitor glucose concentration stably.

In this paper, we propose an implantable flexible glucose sensor based on a parylene sheet that can detect the fluorescence of the glucose-responsive hydrogel directly and continuously. (Fig. 1). Parylene has been widely used material for a substrate of implantable device because its biocompatibility and flexibility allows deforming as patients move. We fabricated a parylene based flexible electrode with a LED chip and a photodiode, and formed a glucose-responsive hydrogel on it. This device can deform flexibly and measure the fluorescence intensity subcutaneously. We achieved glucose monitoring continuously in vitro changing glucose concentration from 0 to 300 mg/dL. We also demonstrated implantation of the parylene based flexible glucose sensor into a rat and measured glucose concentration continuously at its interstitial fluid.

FABRICATION AND METHOD

Fabrication of flexible electrode

The flexible electrode was fabricated by a standard photolithography process. First, Cu sheet (10 μm thickness) was dipped in silane solution (C_2H_6O: H_2O: silane = 100:100:1) over 30 min for silane coupling to Cu. After putting the Cu sheet on a silicone film (Fig. 2(a)), gwe deposited 10 μm thick parylene-C (Cookson Electronics Co.) on it by a parylene vapor deposition machine (LABCOATER PDS2010, Speciality Coating Systems, USA) (Fig. 2(b)). Subsequently, we peeled the Cu sheet with parylene-C from the silicone film and taped the sheet upside down on a glass plate (Fig. 2(c)). Next,

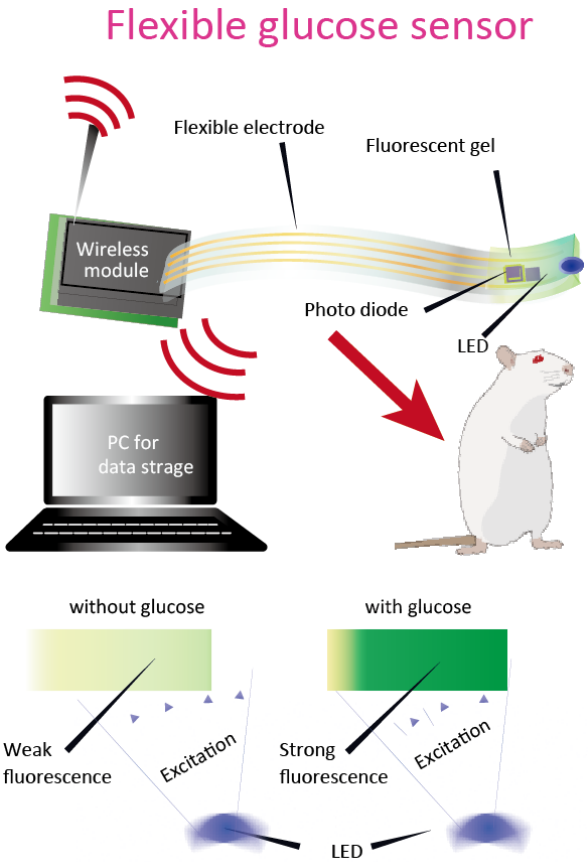


Figure 1: Concept of a parylene based flexible glucose sensor. A blue light under glucose existing situation excites glucose-responsive fluorescent hydrogel. Emitted green fluorescence from the hydrogel is detected by a photo diode. The of the photo diode is continuously transmitted to a computer through a wireless module. Thus, the device enables us to monitor glucose level by measuring fluorescence intensity.

S1818 (Rohm and Haas Company) was spincoated on the Cu sheet at 3000 rpm for 30 sec. We baked the sheet at 100 °C for 5 min to cure the photoresist and then we exposed the sheet with S1818 layer to UV light for 12 sec through a glass mask. We dipped the sheet into NMD (tetramethylammonium hydroxide, Tokyo Ohka Kogyo Company) for 60 sec to etch the S1818 layer (Fig.2 (d)). Subsequently, we etched the Cu sheet by Cu etchant in order to make electrodes on the parylene-C sheet and removed the S1818 layer by acetone to obtain a flexible

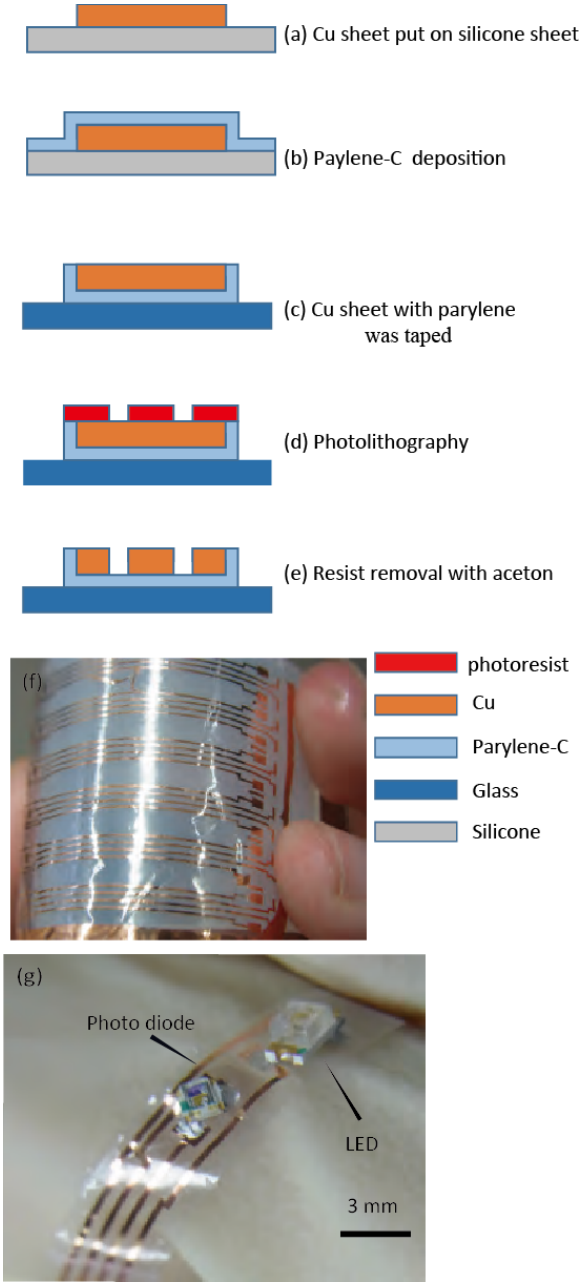


Figure 2: Fabrication process of the flexible glucose sensor. (a) Cu sheet (10 μm thickness) coated with silane coupling was sticked to a silicone sheet. (b) Parylene-C was vapor deposited on the Cu sheet. (c) Cu with parylene-C was peeled from the silicone sheet and taped upside down on a glass plate. (d) S1818 is patterned using photolithography. (e) Cu was etched by wet etching and photo resist was removed by acetone. (f) Fabricated a parylene-C sheet with patterned Cu. (g) A LED and a photo diode chip were mounted on a flexible sheet with circuit pattern.

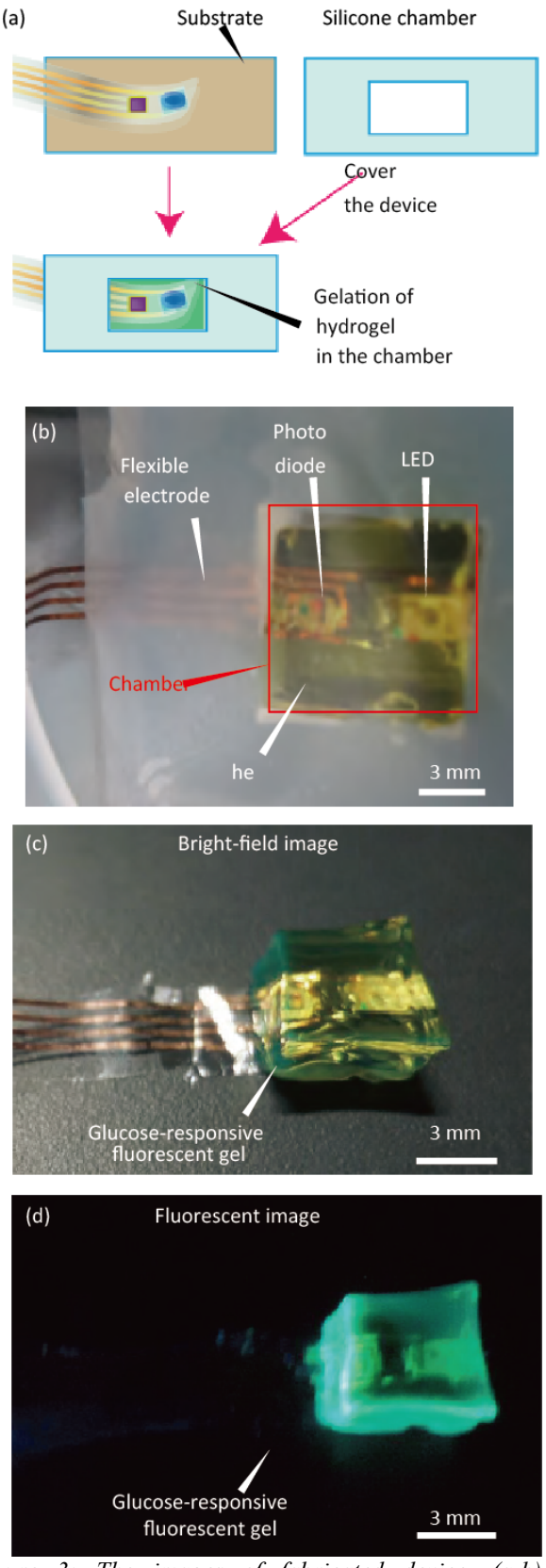


Figure 3: The images of fabricated device. (a-b) Glucose-responsive hydrogel was poured into a silicone chamber. (c-d) The flexible glucose sensor (c) under room light and (d)Glucose-responsive hydrogel generate fluorescence under blue light in a glucose solution.

electrode (Fig. 2(e, f)). After fabrication of the flexible electrode, we mounted a LED chip (SM1206UV-405-IL, Biver Company) and a photo diode chip (APDS-9005-020, AvagoTechnologies Company) on the electrode by solder (Fig. 2(g)). After mounting the LED chip and photo diode chip on the electrode, we deposited 2 μm thick parylene on it to protect the Cu electrode and these chips.

Formation of glucose-responsive fluorescent hydrogel

We set the parylene based flexible electrode on a silicone substrate and then put the silicone sheet based chamber (8 mm×8 mm×2 mm) on the flexible electrode (Fig. 3(a)). After setting the flexible electrode under the chamber, we mixed 15w/v% acryl-amide (Wako Pure Chemical Industries Ltd.), 0.3 w/v% *N, N'*-methylene-*bis*-(acrylamide) (Wako Pure Chemical Industries Ltd.i), a 60 mM phosphate buffer with 1.0 mM ethylenediaminetetraacetic acid (Nacalai Tesque Inc) and 5 w/v% Poly (ethylene glycol) methyl ether acrylate (Aldrich). Then, we add 0.36 w/v% sodium persulfate (SPS) (Kanto Chemical Co. Inc.) and 1.6mM *N, N, N′, N′*-Tetramethylethylenediamine (Wako Pure Chemical Insustries Ltd.i), to the pre gel solution and poured them into the chamber and formed the glucose-responsive hydrogel covering the LED chip and the photo diode chip on the electrode(Fig.3 (b)). The chamber containing pre gel solution was sealed with a PET film in order to prevent an inflow of oxygen, and heated at 37 °C for 30 min to cure the hydrogel. After that, we washed device with distilled water to wash out pre gel solution because acrylate monomer cause inflammation. We preserved the glucose-responsive hydrogel in the water to avoid the hydrogel drying.

In vitro glucose monitoring test

We used the photo diode to detect the change of the fluorescence intensity of glucose-responsive hydrogel excited by the LED (405 nm). The device was programmed to turn on the LED every 1 min, and then read and transmit the data of the photo diode to a personal computer through the wireless transmission module. We conducted this process 30 times each and plotted the change ratio of the fluorescence intensity (Fig. 4). We used the value of intensity at 0 mg/dL as the reference f_0

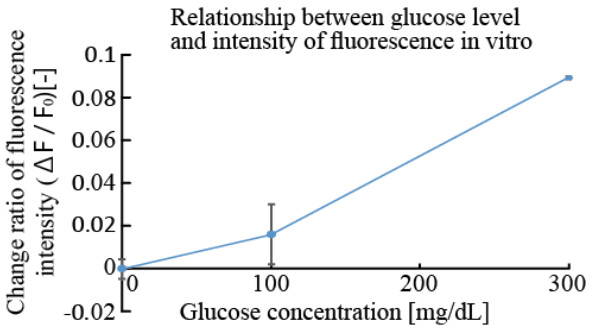


Figure 4: Plot of fluorescence intensity detected by the photodiode depending on glucose concentration from 0 to 300 mg/dL. The intensity of fluorescence increased as the concentration of glucose became higher.

and calculated the change ratio of fluorescence as

$$\Delta f/f_0 = f_i/f_0 - 1 \quad (1)$$

Here, f_i (i =0,1,2) is the average intensity of fluorescence at each concentration

In vivo glucose monitoring test

First, we dissected its dermis at its back, put the parylene based flexible glucose sensor on its fascia, and sutured its back under anesthetic (Fig.5 (b)). The flexible glucose sensor was able to deform along with the body of the rat. Then, we fixed the wireless module and a battery on its back to transmit the fluorescence intensity of the gel to the computer.

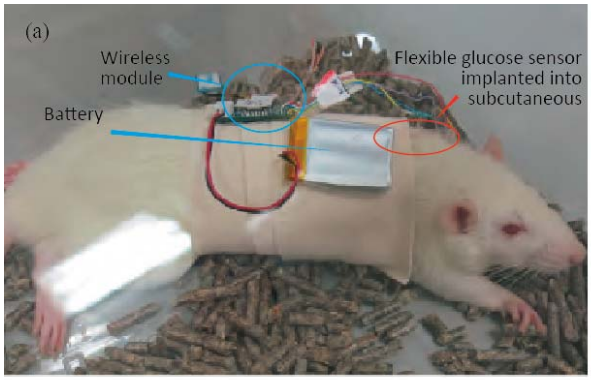
RESULTS AND DISCUSSION

Fabrication of the flexible glucose sensor

Figure 3(c) was an image of fabricated parylene based flexible glucose sensor (in blight field). We confirmed fluorescence of the glucose-responsive hydrogel on the device in glucose solution under blue light condition (Fig. 3 (d)).

In vitro glucose monitoring test

We tested response of glucose-responsive hydrogel in vitro. We changed the concentration of glucose solution from 0 to 1000 mg/dl. The fluorescence intensity measured by the flexible glucose sensor increased as the glucose concentration became higher.



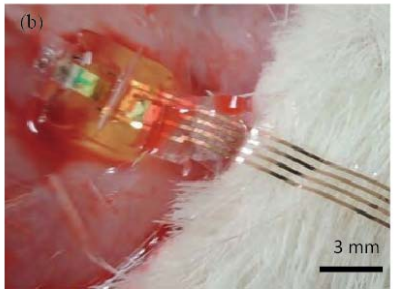


Figure 5: Monitoring in vivo glucose level using the flexible glucose sensor. (a) Image of set up for implantation of the flexible glucose sensor. Red circle shows the flexible glucose sensor implanted into subcutaneous area of a rat and blue circle shows the wireless data transmission module. (b) The flexible glucose sensor on back of a rat. (c) Change ratio of fluorescent intensity in the rat under general anesthetic.

In vivo glucose monitoring test

We implanted the flexible glucose sensor into the back of a rat and conducted in vivo glucose tolerance testing (Fig. 5(a)). The flexible glucose sensor with glucose-responsive hydrogel were inserted to the back of rat deforming along with the body (Fig. 5(b)). We measured the glucose concentration of interstitial fluid that is known to reflect the blood glucose concentration [9, 10].

After implanting the flexible glucose sensor, we achieved measurement of the glucose concentration at interstitial fluid more than 1 hours.

CONCLUSIONS

We fabricated the parylene based glucose sensor with glucose-responsive hydrogel. We measured the change of the fluorescence intensity in vitro as the glucose concentration increase. We demonstrated the implantation of the flexible glucose sensor into a rat and achieved continuous glucose monitoring in interstitial fluid. In the future work, we will replace these surface mount LED chip and photo diode chip with bare chips to make the flexible glucose sensor smaller.

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