In vitro Evaluation of the Continuous Monitoring Glucose Sensors with Perfluorinated Tetrafluoroethylene Coatings

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In vivo monitoring with implanted probes has been steadily studied in recent decades.[1-7] For clinical uses, the implanted probes should meet strict requirements such as outstanding reliability, stability and low interference.[4] Very recently, a few successful sensors were characterized[8-13] and commercialized.[14-20] Now, mass production has become the keyword in this field.[21]

In the previous work, it was demonstrated that the ultra-thin bilayer structures, in which both the inner and enzyme layers were electropolymerized consecutively, had valuable advantages over other types of enzyme electrodes for the minimally invasive needle type glucose sensors.[22-24] One of the strong merits is that the whole fabrication process of the extremely thin chemical structures is carried out in aqueous media. It offers an opportunity to reduce the probability of critical damages in enzyme activities due to the organic solvents that are used during the conventional casting process. Moreover, it requires less caution to maintain the high reproducibility of the electropolymerized membranes because the characteristics of the thin layers depend only on the variables that are easily and precisely controllable with voltage applied, the concentration of monomers, and electrolysis time. However, even though such bilayer systems are suitable for the miniaturized enzyme electrodes, it might be useless without any outer layer that not only possesses excellent biocompatibility but also allows easy casting.

In this study, we have suggested perfluorinated tetrafluoroethylene (PTFE) film cast from aqueous media as the outer membrane. The cross-sectional chemical structure of the glucose sensors suggested in this work is depicted in Figure 1. The ultra-thin inner membrane is poly-5-amino-1-naphthalene(poly-5A1N), on which poly-1,3-phenylene diamine (poly-*m*-PD) and enzymes was simultaneously electropolymerized.[23,24] On the top of the bilayer, PTFE was overlaid as a hydrophobic and biocompatible outer membrane. The present paper reports *in vitro* evaluation of the glucose sensors with this novel tri-layer structure.

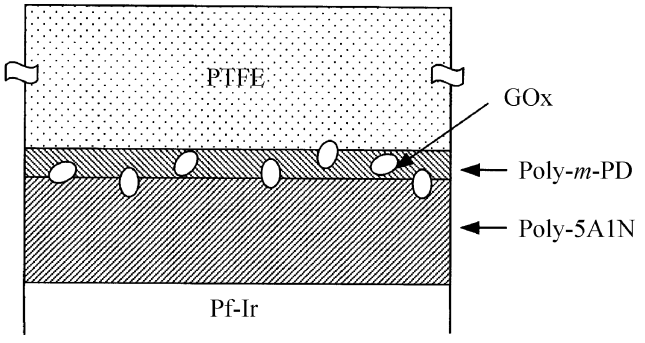

Figure 1. Schematic view of the trilayered glucose sensor. The thicknesses of poly-5A1N, poly-*m*-PD and PTFE are *ca.* 24 nm, 8 nm and 4 μm, respectively.

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Results and Discussion

The electropolymerized bilayer containing glucose oxidase, GOx, (Pt-Ir\poly-5A1N\poly-*m*-PD+GOx) suffers from negligible interference by ascorbic acid (AA) and acetaminophen (AP), as shown in Figure 2(A). Therefore, any further selective property is required for the outer layer. The outer membrane on the Pt-Ir\poly-5A1N\poly-*m*-PD+GOx was cast from an aqueous solution in which PTFE is homogeneously dispersed. The thickness of the PTFE outer membrane was *ca.* 4 μm, which was much thicker than the inner and enzyme layers. The surface morphology of the PTFE membranes were confirmed by scanning electron microscope (SEM). The SEM images before and after the *in vitro* tests in human serum media were hardly distinguishable. Neither apparent damage of the PTFE surfaces nor failure of the

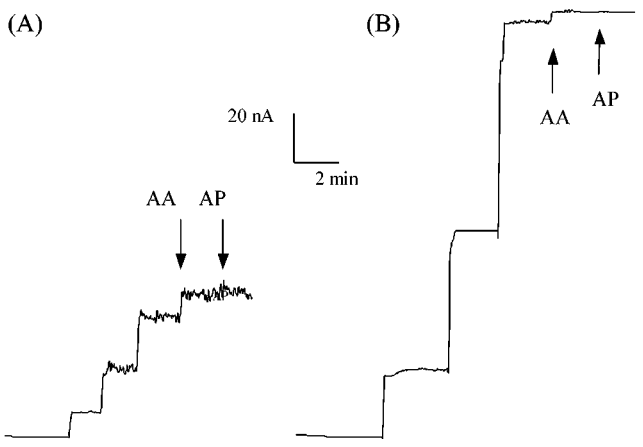

Figure 2. The current responses of Pt-Ir\poly-5A1N\poly-*m*-PD+GOx (A) and Pt-Ir\poly-5A1N\poly-*m*-PD+GOx\PTFE (B) to 6 mM glucose, 0.11 mM AA and 0.17 mM AP in PBS. The concentrations of AA and AP are the physiological maximum.

functions as sensors were observed after more than ten times experiments in the convective phosphate buffered saline (PBS) solutions.

With the PTFE membrane, the sensitivity remarkably increased while the electropolymerized bilayer was still highly selective as shown in Figure 2(B). Two possible reasons can be suggested. First, in the absence of the thick outer membrane H_2O_2 generated in the enzyme layer is likely to move outwards and dissipates into bulk solution because the diffusion coefficient in poly-5A1N is supposed to be far lower than water. Hence the diffusion flux of H_2O_2 toward bulk solution is much higher than that toward the Pt-Ir electrode surface. It is believed that the presence of the outer membrane blocks the loss of H_2O_2. The increased flux toward the electrode surface boosts the concentration of H_2O_2 in the poly-5A1N layer, which results in the enhancement of the faradaic current. The mathematical simulation of an enzyme electrode with almost the same membrane structure is well consistent with this scenario.[25,26] Second, the PTFE membranes cast from aqueous solutions can stabilize the enzymes by being interposed between the enzyme layer and the bulk solution and thereby protecting the inner layer from mechanical damages or loss of enzymes. Presumably, this thick biocompatible outer membrane plays both roles of a good mechanical support and a layer providing with an environment favorable for enzymes.

The apparent Michaelis-Menten constants (K_m') of Pt-Ir\poly-5A1N\poly-*m*-PD+GOx and Pt-Ir\poly-5A1N\poly-*m*-PD+GOx\PTFE from the Eadee-Hofstee plot[27,28] of rotating-disk electrode experiments are 18.8 mM and 10.6 mM, respectively. The significant decrease of K_m' in the presence of PTFE agrees with both reasons that are suggested above.

Table 1 summarizes the results of *in vitro* tests in PBS with 9 sensors random-sampled from a batch production. The background current reproducibly reached a steady state within 10 min. Once the current was stabilized, no fluctuation larger than 0.1 nA was observed. The sensitivity is under control by adjusting the thickness of PTFE, which varies linearly with the number of dipping in aqueous PTFE solution. Drying at a reduced pressure (*ca*. 0.5 mmHg) in a vacuum oven improves reproducibility and stability. Moreover, the time for drying process is remarkably saved. The sensors respond to the changes in the concentration of glucose so rapidly that t_{90} is less than 1 s. The signals by the representative electro-active species in a physiological medium are less than 1% of that from 6 mM of glucose, which is the normal physiological level.

Table 1. Results of *in vitro* test with the miniaturized glucose sensors

	Results (n = 9)[a]
Background current	3.5 nA/mm^2 [b]
Normalized sensitivity	3.9 ± 0.4 nA/mM mm^2 [c]
Response time (t_{90})[d]	< 1 sec
Linear response range	0-30 mM (R^2 = 0.98) 1-9 mM (R^2 = 0.99)
Interference[e]	
ascorbic acid	< 1%
acetaminophen	< 0.7%
uric acid, urea, L-cysteine, D(−)fructose	Not detected

[a]Independently fabricated electrodes. [b]The deviation is within reading error range that is less than 0.1 nA. [c]Measured the concentration range from 1 to 9 mM. [d]Time required for the signal to reach 90% of the steady state current. [e]Relative ratios of interferences by physiological maximum of potential interferents in the presence of 6 mM glucose solution.

Another important requirement for continuous monitoring sensors is the minimal hysteresis. The hysteresis of Pt-Ir\poly-5A1N\poly-*m*-PD+GOx\PTFE is less than 1.6 nA corresponding to *ca*. 0.4 mM in the range of 1-9 mM glucose. Considering that glucose level in normal blood is 4.4-6.6 mM, the maximal error of 0.4 mM is small enough to detect abnormalities in glucose concentration. The loss of sensitivities does not exceed 10% after the everyday tests in whole blood for 2 weeks during which the sensors are rinsed with PBS and stored in dried chamber.

The effect of oxygen is also crucial for the glucose sensor in this study because GOx continuously consumes oxygen and may cause local oxygen depletion. As the partial pressure of O_2 is reduced from 150 mmHg to 5 and 15 mmHg, the sensitivity decreases by 30.0% and 15.8%, respectively. On the other hand, the sensitivity is lowered by 29.3 % in the 1-6 mM range of glucose in five-fold diluted human serum samples compared with that in PBS. The sensitivity decay in serum is not negligible but its extent is reproducible so that it could be corrected by calibration. It is expected that the outer membrane relieves the problem in such sensitivity drop in serum and oxygen-deficient media. Perfluorinated polymers reportedly help with removing oxygen dependence.[29] Optimization of the composition and thickness of outer membrane and results from *in vivo* tests will be published in a separate paper.

Experimental Section

The experimental setup and conditions for the fabrication and characterization of the bilayered enzyme electrodes, Pt-Ir\poly-5A1N\poly-*m*-PD+GOx, were described in the previous paper.[24] A dispersed perfluorinated tetrafluoroethylene in water (60%, PTFE solution) from Aldrich was used without further purification. A Pt-Ir\poly-5A1N\poly-*m*-PD+GOx was dipped into dispersed PTFE solution diluted to 30% for 5 sec. The dipping process was repeated five times before drying the electrode under a reduced pressure *ca.* 0.5 mmHg at 30 °C for 15 min. After repeating the dipping/drying procedure five times, the finalized sensor was equilibrated in PBS for 1 h. The glucose sensor, to which a constant potential is applied to measure the current, was placed in PBS continuously stirred at a fixed speed until the background current reached a stabilized level. It takes less than 10 min to reach a stable, reproducible current. The ratio of the current due to maximum physiological concentration of interferents (i_{int}) to that due to 6 mM glucose (i_g) was used to calculate the interference, ($i_{int}/i_g \times 100\%$). The maximum physiological concentration of ascorbic acid

(AA), acetaminophen (AP), uric acid, L-cysteine, D(–) fructose are 0.11, 0.17, 0.48, 0.1 and 0.4 mM, respectively. The effect of O_2 tension was observed by obtaining the sensitivity to glucose after feeding O_2-N_2 mixed gas into the test solution. The samples of human serum were donated by The Seoul National University Hospital and the glucose concentrations of those were independently measured with a Hitachi Automatic Analyzer 747-100 (Hitachi, Ltd. Instrument Division 882, Ichige, Hilachinaka-ahi, Ibaraki-ken, 312-8504 Japan). The serum samples were diluted to 20% v/v with PBS. To examine hysteresis of the signal, the glucose concentration in the test cell was raised by adding the stock solution of glucose and reduced by taking out calculated volumes of the solution from the cell and adding pure PBS. The glucose concentration was switched between 4 and 7 mM more than 9 times. All experiments were carried out in PBS at 37 °C, which was kept constant by a water-circulated thermostat (Model B, Lauda-Königshofen, Germany).

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