A porous microneedle electrochemical glucose sensor fabricated on a scaffold of a polymer monolith

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Abstract

Porous microneedle electrodes with pores of ∼1 μm diameter were fabricated by electroless plating of nickel followed by gold on a polymer monolith of poly(glycidyl methacrylate). The specific surface area of the fabricated electrode evaluated by the Brunauer–Emmett–Teller method was 2.559 ± 0.050 m² g⁻¹ (standard error of mean), while that of the non-porous control was <0.001 m² g⁻¹. Electrochemical glucose sensors were then fabricated by immobilizing glucose oxidase on the gold-plated microneedle electrodes. The sensitivity of the porous microneedle glucose sensor between 0 and 15 mM glucose was 22.99 ± 0.72 µA mM⁻¹, and that of the non-porous control was 3.16 ± 0.56 µA mM⁻¹. The amperometry of glucose concentration in solution was demonstrated using the fabricated electrode as a working electrode, along with an Ag/AgCl reference electrode and gold counter electrode both of which were made of microneedles. These results indicate the advantages of porous structures for electrochemical sensing with increased sensitivity.

1. Introduction

The microneedle array (or microneedles), a projected array of small needles with the height of several hundreds of micrometers, has been originally developed as apparatus for minimally invasive drug delivery [1]. Microneedles can penetrate stratum corneum, the outermost layer of skin, in a minimally invasive manner, and deliver the drugs into skin interstitial fluid in epidermis, which is eventually diffused into capillary vessels and the entire body. In the past decade, along with the rise of epidermal electronics and transdermal biosensing [2, 3], microneedles are also actively studied as a tool for sampling [4, 5] and analysis [6] of skin interstitial fluid. For example, electrochemical sensing of glucose by microneedles has been studied [6–9]. Glucose concentration in skin interstitial fluid is correlated with that in blood, and measurement of glucose concentration in skin interstitial fluid by microneedle sensors may become a possible alternative to a conventional invasive method for glucose monitoring of patients with diabetes. Nishizawa’s group has pioneered the development of porous polymer microneedles made of a polymer monolith of poly(glycidyl methacrylate) (PGMA) having interconnected macropores [10]. The macropores were spontaneously formed by phase separation during polymerization of glycidyl methacrylate and bi- and tri-functional crosslinkers in the presence of polyethylene glycol as a porogen (sacrificial template of pores) [11]. The developed porous polymer microneedles have potential for rapid and high-volume intake of skin interstitial fluid due to capillary action, and they were applied to the ionic conduction between skin and wearable batteries and external devices [12, 13]. Other materials than polymers have also been used to fabricate porous microneedles. Cahill et al fabricated metallic porous microneedles by sintering stainless steel powder, and tested their capability for insertion into skin and model drug release [14].

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Electrochemical sensors rely on electrochemical reactions of surface-bounded enzymes and other molecules that are selective to the analyte, which occurs at the interface between the electrode and solution. Therefore, the larger surface area of the electrode leads to higher sensitivity, the intensity of an electric signal per unit concentration of the analyte. Porous metals have been studied as highly active catalysts as well as sensitive electrodes because of their large surface areas. However, such porous metal electrodes have not been utilized for microneedle sensors. In this study, we fabricate porous microneedle electrodes by electroless plating of nickel and gold on the porous polymer microneedles, and applied them to an enzymatic glucose sensor (figure 1). Electroless plating processes maintain the original interconnected macroporous structures of the microneedles, and outer and inner surfaces can be coated with gold, which leads to the microneedle electrodes with a high specific surface area.

2. Materials and methods

HEMA was purchased from Tokyo Chemical Industry Co., Ltd and the polymerization inhibitor was removed by an inhibitor remover (Sigma-Aldrich) prior to synthesis of poly(vinylferrocene-co-2-hydroxyethyl methacrylate) (poly(VF-co-HEMA)). Silver chloride ink was purchased from ALS Co., Ltd. Dehydrated dichloromethane and other reagents were purchased from FUJIFILM Wako Pure Chemical Corporation and used without further purification. Proton nuclear magnetic resonance (1H-NMR) was measured on Bruker model AVANCE III 400, and SEC-HPLC was measured on JASCO model LC-4000LG with a column KF-805L (Showa Denko) and a UV detector at 250 nm wavelength.

2.1. Fabrication of porous polymer microneedles

Porous polymer microneedles made of PGMA were fabricated according to the previous literature. A mixture of glycidyl methacrylate, trimethylolpropane trimethacrylate, triethylene glycol dimethacrylate, and polyethylene glycol (molecular weight: 10 kDa) in methoxyethanol was poured in a polydimethylsiloxane (PDMS) mold, and irradiated with UV by a UV lamp (365 nm; Analytik Jena UVL-28) for 1 h. The polymerized microneedles were peeled off from the mold, and washed in ethanol/water (1:1 volume ratio) at 50 °C for 1 h three times.

2.2. Electroless plating of nickel and gold on porous polymer microneedles

The porous polymer microneedles fabricated above were subjected to electroless plating with nickel by the method that was modified from the previous report. The microneedles were immersed into 10 wt% aqueous solution of ethylene diamine for 1 h at room temperature. The resulting specimen was treated with 0.1 wt% palladium chloride solution in 1.0 wt% HCl aq. for 1 h. After that, palladium was reduced by 0.10 wt% sodium phosphinate aq. for 1 h, and treated with an aqueous mixture of nickel chloride (0.050 M), trisodium citrate dihydrate (0.13 M), ammonium chloride (0.38 M), and sodium phosphinate (10 wt%) at 80 °C for 2 h. The resulting nickel-plated microneedle array was treated in an electroless gold plating bath (Muden Noble AU, Okuno Chemical Industries Co., Ltd) at 65 °C for 2 h. The specimen was washed by stirring in distilled water for 1 h between each step.

2.3. Surface area measurement

The Brunauer–Emmett–Teller (BET) surface areas of microneedle specimens were measured on QUADRASORB EVO4 with nitrogen gas as absorbate. The same set of specimens was subjected to one
isotherm cycle to remove surface-bound water and other volatile molecules, followed by two measurement cycles to calculate mean and standard error of mean of the surface area.

2.4. Synthesis of 1-hydroxyethylferrocene
1-Hydroxyethylferrocene was synthesized following a method in the past literature [18]. In a 300 ml beaker in air, acetylferrocene (6.124 g, 26.85 mmol) was dissolved in methanol (150 ml), and sodium borohydride (5.048 g, 133.4 mmol, 5.0 equiv.) was added in one portion under stirring. The reaction was highly exothermic and bubbles were vigorously produced. After 20 min, the reaction mixture was poured into 200 ml water, and it was extracted three times with 200 ml, 150 ml, and 100 ml of dichloromethane. The combined organic layer was washed with brine (200 ml), dried over sodium sulfate, and evaporated to dryness. The crude product was characterized by $^1$H-NMR. No byproduct was observed. $^1$H-NMR (CDCl$_3$, 400 MHz): $\delta$ 1.44 (d, 3H), 1.84 (d, 1H), 4.20 (s, 5H), 4.17 (m, 2H), 4.22 (m, 2H), 4.56 (m, 1H).

2.5. Synthesis of poly(VF-co-HEMA)
Synthesis of poly(VF-co-HEMA) was carried out in accordance with the literature method [18]. To 1-hydroxyethylferrocene (5.947 g) in a 200 ml round bottom flask cooled with an ice bath under argon flow was added dimethylaminopyridine (159 mg), dehydrated dichloromethane (60 ml), triethylamine (10.8 ml), and methanesulfonyl chloride (1.338 ml). The reaction mixture was stirred under nitrogen flow at room temperature for 6 h. Dichloromethane was poured into the flask to adjust the solution volume to 60 ml, and 5.0 wt% NaHCO$_3$ solution (120 ml) was added to the mixture to quench the reaction. The organic layer was collected, and the aqueous layer was extracted with 0.1 l of dichloromethane three times. The combined organic layer was washed with 0.3 l of brine, dried over sodium sulfate, and evaporated to dryness. The resulting crude oil was subjected to column chromatography on activated basic alumina (Wako). The first colored fraction was collected and evaporated to dryness to obtain red-brown oil. The resulting product was characterized by $^1$H-NMR. $^1$H-NMR (CDCl$_3$, 400 MHz): $\delta$ 1.41 (s, 5H), 4.21 (m, 2H), 4.35 (m, 2H), 5.02 (dd, 1H), 5.33 (dd, 1H), 6.45 (dd, 1H). The NMR spectrum indicated ~30% of unidentified impurity without a vinyl group. The obtained product was used for the next reaction in the presence of this impurity.

2.6. Synthesis of a polymer mediator
Polymer mediator poly(VF-co-HEMA) was synthesized by following the procedure reported in the literature [19]. In a 30 ml Schlenk tube, vinylferrocene (2.20 g, 10.4 mmol), HEMA (1.88 ml, 15.5 mmol), benzene (1 ml), and azobisisobutyronitrile (AIBN) (22 mg, 0.13 mmol) were mixed under stirring. The reaction mixture was degassed by five freeze-pump-thaw cycles, and the flask was purged with argon. The reaction mixture was heated at 60 $^\circ$C for 25 h with stirring, although the reaction mixture became solid and the stir bar was stuck 12 h after heating started. The solidified reaction mixture was dissolved in N,N-dimethylformamide (DMF) and reprecipitated in benzene twice. The resulting sticky red-brown solid was washed with water and dried under vacuum. Red-brown solid was obtained (1.42 g). Average molecular weight was determined by size exclusion chromatography on a column Shodex KF-805L with tetrahydrofuran as eluent on JASCO model LC-4000LG. Calibration was carried out with polystyrene standards. $M_n = 3.2 \times 10^4$ g mol$^{-1}$, $M_w = 7.3 \times 10^4$ g mol$^{-1}$.

2.7. Fabrication of the porous microneedle electrode modified with glucose oxidase
The gold-plated microneedles were immersed in aqueous cystamine dihydrochloride solution (10 mM) followed by glucose oxidase solution (20 µM in 0.10 mM pH 7.0 phosphate buffer solution) whose hydroxyl groups in the saccharide chains were oxidized to aldehyde beforehand [20]. To fabricate the reagentless microneedle glucose electrode with an immobilized polymer mediator, the gold-plated microneedles were first immersed into aqueous cystamine dihydrochloride solution (10 mM) for 1 h and washed with water. The cystamine-modified microneedles were then immersed into solution of poly(VF-co-HEMA) in DMF (10 wt%) followed by drying at 65 $^\circ$C overnight. The dried specimen was treated with glucose oxidase solution (20 µM in 0.10 mM pH 7.0 phosphate buffer solution) to obtain the reagentless microneedle glucose electrode.

2.8. Electrochemical measurement
Electrochemical measurement was conducted on a potentiostat/galvanostat model ECstat-301WL (EC Frontier, Co., Ltd). Cyclic voltammetry (CV) of the fabricated glucose oxidase electrode was measured with a scan rate of 10 mV s$^{-1}$ in 0.10 M pH 7.0 phosphate buffer solution with 0.1 mM ferrocene methanol and various concentrations of glucose with Pt as a counter electrode and Ag/AgCl as a reference electrode. The amperometric responses of the microneedle glucose electrode were evaluated by chronocoulometry at 0.3 V or 0.4 V (vs Ag/AgCl) during the successive addition of glucose to 0.10 M pH 7.0 phosphate buffer.
solution with or without 0.1 mM ferrocene methanol. Response time was defined as the time required for the electrode to undergo 90% of the total change upon the addition of glucose. All measurements were conducted under stirring with a magnetic stir bar rotating at approximately 200 rpm.

3. Results and discussion

3.1. Characterization of gold-plated porous microneedles
The original porous polymer microneedles showed opaque white color as observed by optical microscopy (figure 2(a); left top and bottom). After the electroless gold plating, the microneedles showed uniform, matt gold color (figure 2(a); right top and bottom). This suggested the surface of macropores were sufficiently covered with gold. Scanning electron microscopy—energy-dispersive x-ray spectroscopy (SEM-EDX) measurement also indicated that the surface was covered by gold (figure 2(b)). The specific surface area of pristine porous microneedles (before gold plating) with 54% porosity was 2.319 ± 0.011 m$^2$ g$^{-1}$. After gold plating of the porous microneedles, the specific surface area slightly increased to 2.559 ± 0.050 m$^2$ g$^{-1}$. This can be attributed to the rough nature of the gold-plated surface, as observed by SEM (figure S1 (available online at stacks.iop.org/JPENERGY/3/024006/mmedia)). On the contrary, the specific surface area of the gold-plated, non-porous microneedles was below the limit of measurement by BET isotherms (<0.001 m$^2$ g$^{-1}$).

3.2. Amperometric glucose sensing by the microneedle glucose electrodes
Glucose oxidase was immobilized by first partially oxidizing the sugar chain to aldehyde and coupling it to the cystamine-modified gold surface. After the immobilization of glucose oxidase, CV on the microneedle glucose electrode was measured in the presence and absence of glucose in solution (figure 3). The electrode showed increased current response to the increased concentration of glucose in solution, and the CV was reversible over four cycles, which indicated the stability of covalently attached glucose oxidase on the gold surface during repeated redox reactions. The sensitivity of porous microneedle electrodes between 0 and 15 mM was evaluated to be 22.99 ± 0.72 µA mM$^{-1}$ (standard error of mean) (figure 4). On the contrary, that of the non-porous control was 3.16 ± 0.56 µA mM$^{-1}$. The response time (time required for 90% of the
Figure 3. (a) Cyclic voltammetry of the glucose electrode without glucose (blue) and with 20 mM glucose at the first (gray) and fourth (red) cycles in 0.1 M phosphate buffer solution. (b) Cyclic voltammetry for various glucose concentrations. Glucose concentration: 0 mM (blue), 5 mM (orange), 10 mM (green), 15 mM (purple), 20 mM (red).

Figure 4. Amperometric sensing of glucose with the applied potential of 0.3 V (vs Ag/AgCl) by the porous microneedle electrode with an electron mediator dissolved in solution. Conventional Ag/AgCl reference electrode and Pt counter electrode were used. (a) A representative time course of the current of the porous microneedle glucose electrode of 54% porosity during the successive addition of glucose from 0 mM to 20 mM (each step is 1.667 mM increase). (b) Concentration profiles of the microneedle glucose electrodes of 54% porosity (blue) and non-porous ones (orange) (mean and standard deviation of two samples). (c) The sensitivity of the porous and non-porous microneedle glucose electrodes.

The total change (total change) of the porous microneedle electrode was 82 ± 22 s. The larger surface area of the microneedles with higher porosity could contribute to this increased sensitivity, although the difference in sensitivity is not as large as the difference in the surface area. Factors that may affect the sensitivity are coverage of the gold on the surface, thickness of the gold plating, the pore volume distribution, etc. In addition, the effect of internal diffusion in the pores could account for the less pronounced increase in the current.

A phosphate buffer solution is used as a testing solution in the present study, has been used in the previous studies of microneedle sensors \[21, 22\]. However, skin interstitial fluid contains other various ions and therefore, it is useful to evaluate amperometric responses in such an environment to estimate the performance of the sensors in vivo. Artificial interstitial fluid (aqueous solution of 2.5 mM CaCl\(_2\), 5.5 mM glucose, 10 mM HEPES, 3.5 mM KCl, 0.7 mM MgSO\(_4\), 123 mM NaCl, 1.5 mM NaH\(_2\)PO\(_4\), and 7.4 mM sucrose, which was adjusted to pH 7) \[23\] was prepared, and concentration profiles in it were recorded. In the artificial interstitial fluid, the profiles saturated at lower concentration (∼10 mM) than in a phosphate buffer solution, although the profiles in the low concentration range between 0 and 5 mM were nearly identical (figure S2). This result suggested that the ion composition of the solution affected the enzymatic activity of glucose oxidase, but the fabricated electrode is still useful for the measurement of glucose in some concentration range in interstitial fluid.

Immobilization of an electron mediator is important in the development of biosensors that work in situ. In this study, poly (VF-co-HEMA) \[19\] was chosen as an electron mediator between glucose oxidase and the electrode. Poly(VF-co-HEMA) is soluble in DMF but insoluble in water. This difference of solubility...
Amperometric sensing of glucose with the applied potential of 0.4 V (vs Ag/AgCl) by the porous microneedle electrode with an immobilized polymer-type electron mediator. Conventional Ag/AgCl reference electrode and Pt counter electrode were used. (a) A representative time course of the current during the successive addition of glucose. (b) The concentration profile (mean and standard error of mean of two samples).

Experimental setup of the three-electrode system made of all-microneedle electrodes. (b) The response of the three-electrode system to the successive addition of glucose in solution. (c) The representative concentration profile. Applied potential: 0.3 V.

facilitates casting and drying of DMF solution of the polymer mediator on the electrode surface and stable immobilization of the mediator under aqueous conditions. Chronoamperometry of the microneedle electrode with the immobilized poly(VF-co-HEMA) was measured with varying concentrations of glucose in solution (figure 5). The three-electrode system showed a monotonically increasing response of current for varying concentrations of glucose between 0 and 20 mM, although the current saturated around 20 mM. The response time was 103 ± 63 s.

3.3. Amperometric glucose sensing by a three-electrode system with all-microneedle electrodes

Gold-plated porous microneedles without any further modification were used as a counter electrode and solid (non-porous) microneedles coated with silver chloride ink as a reference electrode to perform three-electrode measurement of glucose in solution (figure 6(a)). The testing solutions contained 0.9 wt% NaCl to mimic physiological conditions as well as maintain the constant potential of the reference electrode. In chronoamperometry, the three-electrode system showed the monotonically increasing response of current that saturates around 20 mM glucose (figure 6(b)), which was analogous to that of mediator-modified electrodes. The response time was 8 ± 4 s, which is sufficiently quick for the continuous monitoring of glucose level in skin interstitial fluid in vivo. The reason for the faster response compared to the measurement using the conventional Ag/AgCl reference electrode and Pt counter electrode (figure 5) is most likely due to the variation between samples. It is difficult to precisely control the immobilization process of poly(VF-co-HEMA) mediator, resulting in variations of thickness and morphology of the polymer mediator membrane and the amount of immobilized glucose oxidase, as shown in the difference in electric current between the samples in figures 5 and 6. Changes in the thickness and morphology of the polymer
immobilized on the electrode surface lead to changes in the diffusion rate of glucose as well as ferrocenyl functional groups in the immobilized polymer membrane.

The developed three-electrode system is potentially applicable to a living skin because the three electrodes penetrate skin and make ionic contact between the electrodes and skin interstitial fluid. Nonetheless, several issues need to be addressed to apply the developed microneedle electrodes to actual transdermal biosensing on a living skin. First, the stable ionic contact between the electrode surface and living skin must be maintained, which requires further engineering efforts. Electric current of the sensor depends on the volume of solution with glucose in contact with the electrode surface, and penetration depth affects that volume. Mechanisms to place the microneedle electrode on the skin with fixed penetration depth should contribute to stable electric output. Second, the variation of the electrical output by different temperatures should be considered and appropriately calibrated. Placing the whole sensor in a temperature-controlled chamber or integrating a temperature sensor should be useful to prevent temperature variation or compensate for its effect. In addition, the use of nickel should be preferably avoided for biological safety. Adoption of porous stainless steel or titanium would be a preferable option.

4. Conclusions

In this study, we fabricated the microneedle electrodes having macropores by means of electroless nickel and gold plating on porous polymer monoliths. The fabricated microneedle electrode was further modified with glucose oxidase and an electron mediator to demonstrate glucose sensing in solution. The measurement range covers a part of the concentration range of glucose in skin interstitial fluid of a human body (up to ∼20 mM) [24]. Therefore, the developed glucose sensor is potentially useful for easy and timely monitoring of diabetes. The sensitivity to glucose was improved due to the large surface area of porous structures. The developed porous microneedle electrodes can be a versatile platform for highly sensitive biosensing of the content of skin interstitial fluid. To further increase the sensitivity of the microneedle glucose sensor, the specific surface area should be further increased, e.g. by combining macroporous microneedles with nanoporous conductive materials or metallic nanoparticles that have higher specific surface area.

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