

DEVELOPMENT OF PATTERNABLE NANOPOROUS CARBON ELECTRODES FOR USE AS BIOSENSORS BASED ON REDOX CYCLING EFFECT

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ABSTRACT

This paper reports a highly sensitive electrochemical-enzymatic redox cycling based biosensor platform using patternable nanoporous carbon electrodes. 3D carbon electrodes with macro-/micro-sized pores were fabricated in a batch way using microwave O_2 plasma etching and carbon-MEMS consisting of conventional UV-lithography and pyrolysis. Enzymes (glucose oxidase (GOx) in this study) were immobilized on the nanoporous carbon electrode via diazonium reduction. The enzyme-functionalized nanoporous carbon based sensors exhibited enhanced response for glucose with limits of detection of $216 \mu\text{M}$ and sensitivity of $53.42 \mu\text{M mM}^{-1} \text{cm}^{-2}$ compared to a bare-carbon-based sensor by 2.1 times and 26.0 % respectively.

INTRODUCTION

Diabetes mellitus has recently become one of the most disease leading cause of death worldwide [1-3]. This metabolic disorder results from insulin insufficiency and abnormal glucose concentration (hyperglycemia); the normal glucose concentration ranges from 80 to 120 mg/dL (4.4 – 6.6 mM) [1-5]. Additionally, an increased level of glucose which is associated with diabetes, causes chronic illness and several other complications including higher risks of heart disease, kidney failure, or blindness [4-8]. Therefore, the diagnosis and management of diabetes mellitus need a close monitoring of glucose concentration in blood.

Typical glucose sensing principles for small-sized sensors are enzymatic and non-enzymatic electrochemical detections [9-11]. Non-enzymatic electrochemical detection methods have advantages of fast response, good sensitivity, and simple sensor configuration, but selectivity issue should be overcome for the successful commercialization [9]. Enzymatic electrochemical detection methods enable selective and long-term stable glucose sensing [10]. However, the relatively low sensitivity of the enzymatic electrochemical detection has to be overcome [11]. In the enzymatic electrochemical sensing principle, electrode surface area plays a dominant role in sensing performances because active electrochemical surface and enzyme site affect the electrochemical signal.

In this study, enzymatic-electrochemical current signal is amplified by recycling the redox events of redox mediators (ferricyanide/ferricyanide) between enzyme sites and nanoporous carbon electrode which ensures large surface area (Figure 1a). The nanoporous carbon nanoelectrodes were fabricated using only batch processes including UV-lithography, and subsequent O_2 plasma etching and pyrolysis. Carbon surface modification was progressed from the selective electrochemical reduction of

aryl diazonium salts to GOx immobilization on the carbon electrode surface. Thus, enzymes were immobilized in the proximity of the electrode surface; this enhances the efficiency of redox cycling. In addition, electrochemical signal current was enhanced according to the increase in surface area resulted from the nanoporous electrode surface.

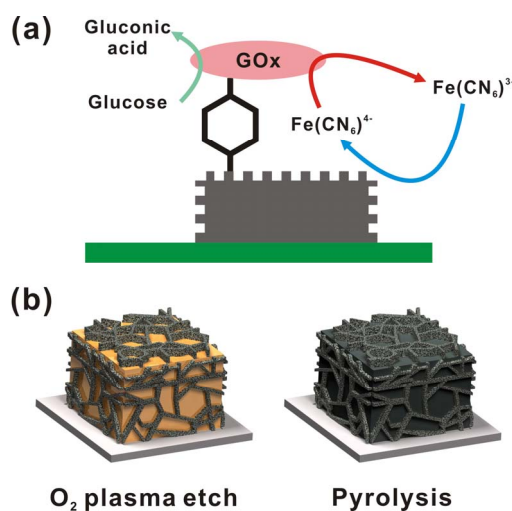


Figure 1: Schematic diagrams of (a) the glucose sensing principle based on redox cycling of $\text{Fe}(\text{CN})_6^{4-}$ and $\text{Fe}(\text{CN})_6^{3-}$ between enzymes and nanoporous carbon surface, and (b) nanoporous carbon electrode fabrication.

EXPERIMENTAL

Fabrication

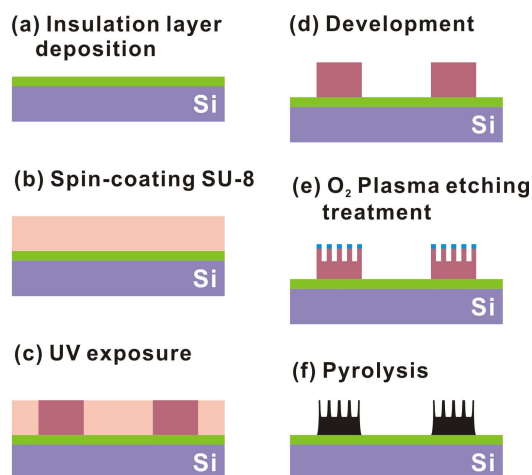


Figure 2: Fabrication steps of patternable nanoporous carbon electrodes

Patternable nanoporous carbon electrodes were

fabricated using carbon-MEMS and O₂ plasma etching processes as shown in Figure 2. Before fabricating carbon structures, an insulating layer of 1- μ m SiO₂ was deposited on a 6-inch Si substrate using wet oxidation. A photoresist (SU-8) layer was spin-coated on the SiO₂/Si substrate and patterned using UV exposure. O₂ plasma etching treatment formed nanopores and wrinkles on the polymer surface. Finally, the nanoporous photoresist structure was converted into the nanoporous carbon structure via pyrolysis at 900 °C in a vacuum condition.

Immobilization of glucose oxidase

The –NO₂ functional groups of the 4-NP units were changed to the –NH₂ groups to selectively attach the enzymes on the nanoporous carbon electrodes. Electrochemical modification on the nanoporous surface was progressed by scanning the electrical potential from 0.5 to -0.7V vs a Ag/AgCl electrode at a scan rate of 200 mV/s in an acetonitrile solution with 1 mM 4-NP diazonium tetrafluoroborate and 0.1 M NBu₄BF₄. After the conversion of –NO₂ to –NH₂, the nanoporous carbon electrodes were linked with glutaraldehyde (Glt) by a dip-coating process in 2 wt% Glt solution and 0.1% NaBH₃CN for 2 hr. Finally, the nanoporous carbon electrodes were incubated overnight at 4 °C in 50 mM PBS buffer with GOx (20 mg/mL) and 0.1% NaBH₃CN to couple the enzyme with the aldehyde groups.

Electrochemical sensing measurement

The nanoporous carbon electrodes were characterized after each step of surface functionalization using cyclic voltammetry in the electrical potential from 0 to –0.8 V vs Ag/AgCl in 10 mM K₄Fe(CN)₆ with PBS solution at a scan rate of 50 mV/s. The counter electrode was a Pt wire.

RESULTS

Surface morphology of nanoporous carbon electrode

Figure 2 (a, c) show the surface morphologies of the SU-8 photoresist structure after the O₂ plasma etching treatment. The sponge-like networks consisting of the nanopore and the wrinkles were formed on the surface of the SU-8 photoresist structure owing to the self-masking effect. Metal ions of the antimony (Sb) included in the SU-8 photoresist play a role of the etch mask to the O₂ plasma etching treatment; this results in the selective polymer etching. In addition, different etch rate between the aromatic and the linear part in the bisphenol A-epichlorohydrin-formaldehyde copolymer also affects the surface morphology of the SU-8 photoresist structure. The morphologies of nanopores such as pore size and density could be controlled depending on the O₂ plasma etching treatment time.

After the pyrolysis process, the sponge-like morphologies on the surface are maintained. However, the volume of the sponge-like morphologies reduced as shown in Figure 3 (b, d). Moreover, 3D nanoporous carbon structures providing more surface area can be easily facilitated on a planar carbon electrode by adding a single UV-lithography process (Figure 3 (e, f)).

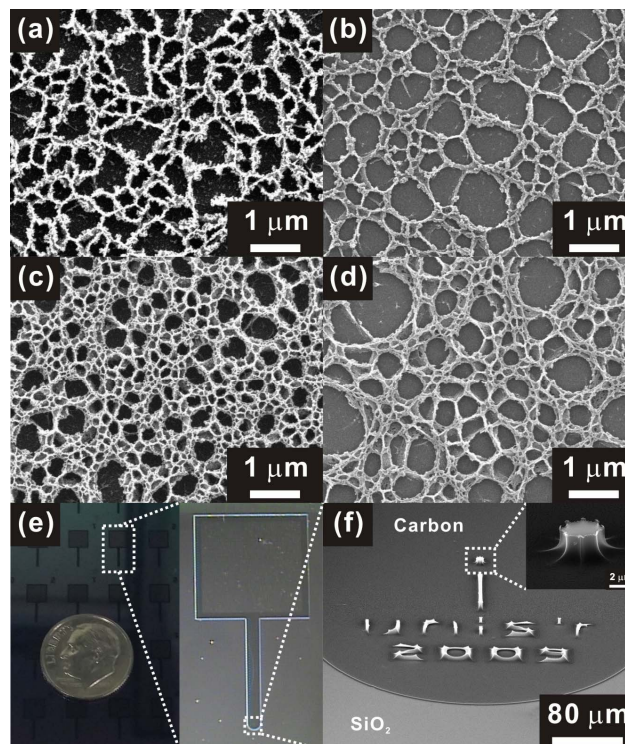


Figure 3: SEM images of (a, c) SU-8 photoresist structures treated with (a) 100 and (b) 200 s plasma etching, and (b, d) corresponding nanoporous carbon electrodes respectively. (e, f) Images of 3D nanoporous carbon structures in the shape of the university emblem “UNIST” on a carbon electrode patterned on the SiO₂/Si substrate.

Electrochemical detection

The nanoporous carbon electrodes were characterized using the cyclic voltammetry after each step of surface modification as shown in Figure 4.

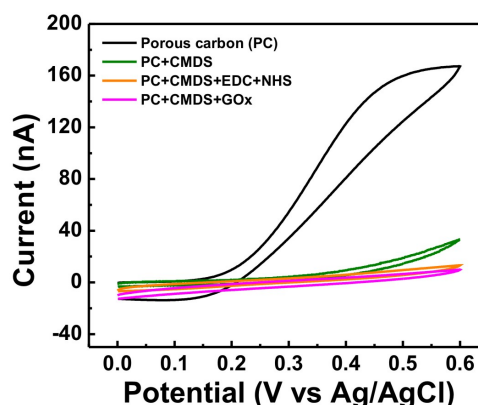


Figure 4: Cyclic voltammograms of the nanoporous carbon electrode from 10 mM K₄Fe(CN)₆ with 50 mM PBS after each electrochemical surface modification step for selectively GOx immobilization.

All electrochemical measurement was performed in the PBS buffer solution with 10 mM K₄Fe(CN)₆. After the electrochemical modification in the 1 mM 4-NP diazonium tetrafluoroborate solution, the electrochemical current collection was blocked because of the hydrophobic surface by the –NO₂ of the 4-NP half group.

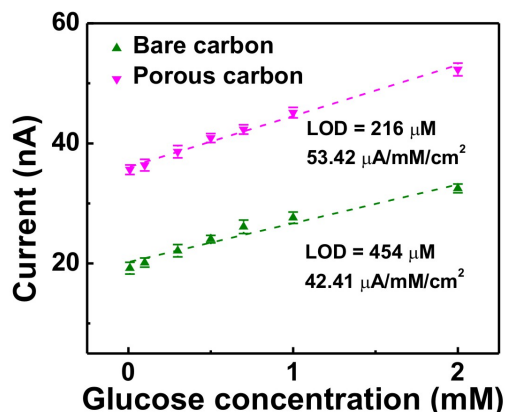


Figure 5: Amperometric current responses from a bare carbon electrode and a nanoporous carbon electrode corresponding to glucose concentration from 10 μ M to 6 mM

The catalytic response of glucose using the nanoporous carbon electrode was characterized using chronoamperometry. The chronoamperometric current were measured using various glucose concentrations from 10 μ M to 6 mM as shown in Figure 5. The enzyme-functionalized nanoporous carbon electrode based sensors showed enhanced response for the detection of glucose with limits of detection of 216 μ M and sensitivities of 53.42 μ M mM^{-1} cm^{-2} compared to bare-carbon-based sensors by 2.1 times and 26.0 % because of enlarged specific surface area, respectively.

CONCLUSION

In this research, we fabricated a highly sensitive electrochemical-enzymatic redox cycling based bio sensing platform using patternable nanoporous carbon electrodes. 3D nanoporous carbon electrodes were fabricated in a batch way using microwave O_2 plasma etching treatment and carbon-MEMS consisting of UV-lithography and the polymer pyrolysis. After the O_2 plasma etching treatment, hierarchical sponge-like nanoporous networks were formed on the surface of the SU-8 photoresist structure owing to the self-masking effect of the metal-ions contained in the SU-8 photoresist. The nanoporous SU-8 photoresist structures was converted to the nanoporous carbon electrode via the polymer pyrolysis. Enzymes were selectively immobilized on the nanoporous carbon electrode via diazonium reduction. Glucose sensing responses of a nanoporous carbon electrode such as the limit of detection and sensitivity were enhanced compared to a bare carbon electrode.

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