Carbon Microstructures for Glucose Biosensor

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ABSTRACT

A novel design and fabrication of glucose sensors based on carbon post electrode array has been reported in this paper. Apart from the fact that carbon has a wide electrochemical stability window, one advantage of using carbon post as working electrode for the amperometric glucose sensor is its high surface to volume ratio, greatly improving sensitivity of glucose sensor consequently. Fabrication process of the high aspect ratio carbon post electrode array and immobilization of enzyme onto the electrodes through electrochemical polymerization of polypyrrole are described in this work. Glucose sensing performance for 140μm, 85μm and 20μm high carbon post with diameter of 30 μm, as well as carbon film without posts are tested and compared. The result demonstrates larger sensitivity with higher posts, which could be explained by more reaction sites due to their larger surface area.

Keywords: Glucose sensor, carbon MEMS, conducting polymer, polypyrrole.

1 INTRODUCTION

The unprecedented interest in the development of analytical devices for detection and monitoring of specific chemical species has led to the emergence of biosensors. Reliable glucose sensing is one of the required technologies in the health care field for monitoring of normoglycemia in diabetic patients suffering from diabetes mellitus. In diabetes mellitus it is now well recognized that the most serious complications, leading to renal failure, blindness, neuropathy and peripheral vascular disease, can be reduced by excess control of blood glucose [1]. On the other hand tight control leads to an increased risk of hypoglycaemia which can also have serious consequences including sudden death. A measure of control is normally achieved through intermittent insulin injections. Regular or continuous blood glucose monitoring is used to assess the level of control.

Glucose oxidase (GOx) is utilized as the biological enzyme in electrochemical transducer of glucose sensors. GOx is known to be an enzyme which can catalyze the electro-oxidation of glucose:

$$\text{Glucose} + O_2 \xrightarrow{\text{GOx}} \text{gluconic acid} + H_2O_2$$

When H_2O_2 comes further to anode surface, the following reaction is carried out:

$$H_2O_2 \xrightarrow{+0.7V} O_2 + 2H^+ + 2e^-$$

When dipped into glucose solution (using PBS as a solvent), the electrode immobilized with GOx can be used as an amperometric glucose-sensitive system. The redox reaction of H_2O_2 near the electrode surface results in a current increase that correlates to the glucose concentration in the solution. Thus by reading anodic current increase, glucose concentration can be deduced.

The stable immobilization of enzyme that would allow for controlled sensing is one of the major problems associated with the manufacture of biosensors. Besides various chemical and physical methods developed for the immobilization of enzymes, progress of research on conducting polymers has led to its use for the immobilization of biomolecules. The electrochemical immobilization of enzymes within conducting polymers has several advantages over conventional methods for the construction of an amperometric biosensor. Firstly, one can control the amount and spatial distribution of enzyme in the polymer in a tailored manner by modification of the charge transferred, as well as monomer and enzyme concentrations in the electrodeposition solution. Secondly, it is also possible to immobilize enzyme on the surface of the electrode by a one-step process regardless of the kinds and shapes of the substrate used for deposition. Finally, the suppression of interference is also expected since a conducting polymer possesses the size exclusion property which is effective in eliminating the interference of electro-oxidizable compounds such as ascorbate and urate. A number of reports on immobilization of glucose oxidase in conducting polymer, particularly PPy, for glucose sensing is available in the literature [2-4]. Polymerization of pyrrole forms an electroactive ion exchange PPy film on the surface of substrate. PPy film helps to form multitude of electroactive centers, thus shortening each electro-transfer paths, and speeding up the electron transfer rate from reaction sites to electrode surface.

The development and the performance of biosensors depend on a large degree on the materials employed for their construction. Ever since Thomas Alva Edison

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discovered that carbon changes its conductivity with pressure and a carbon filament glows when an electric current is passed through it, the unique properties of carbon have intrigued scientists. After more than a century of interest, carbon has found its apogee in a wide variety of familiar materials such as: diamond, graphite, C_{60}, carbon nanotubes (CNTs), carbon nanofibers (CNFs), glassy carbon, and diamond-like carbon (DLC) etc. Structural and functional carbon related materials have a large number of highly specialized applications [5-7]. Complex three dimensional (3D) structures and a wide range of electronic properties from wide bandgap semiconductor (diamond) to metallic (graphite) are possible because of the fact that the coordination chemistry of carbon eases the continuous mixture of single and double -bonded carbon atoms in a structure [8]. The most common electrochemical transducers employed are either inert metals, such as platinum or gold [9,10] or carbonaceous materials [11,12]. Carbon paste as the supporting matrix for the construction of biosensors was researched by many groups earlier attributed to the simple construction procedures required, the low background current it exhibits, its ability for surface regeneration, and its very low cost. Recently Carbon nanotubes have been explored for a wide variety of applications, for example, in bio sensing, where they can alter the catalytic activity and affect the specificity of biological systems [13,14]. Other carbonaceous materials that may be used, as electrochemical transducers are graphite, carbon fibers, porous carbon and glassy carbon. These materials allow for the easy enzyme immobilization while at the same time they possess a wide electrochemical stability window.

In this work we report on a novel design and fabrication of glucose sensor based on carbon post electrode array developed by microfabrication techniques. Glucose sensing performance for 20µm, 85µm and 140µm high carbon post, as well as carbon film are tested and compared. The result demonstrates larger sensitivity with higher posts, which could be explained by more reaction sites due to their larger surface area.

2 EXPERIMENTAL

A typical process flow for fabricating C-MEMS structures is shown in Figure1. In our method, SU-8, a negative tone photoresist is used as the source of Carbon. SU-8 photoresist of desired thickness is spun and photo-exposed creating a pattern of regularly arranged posts. Development was carried out using a SU-8 developer to get rid of the unwanted SU-8 and release the SU-8 microstructures. Photoresist-derived C-MEMS structures were obtained in a two-step pyrolysis process by heating at a high temperature in an inert environment.[15] Madou et al have earlier reported a novel technology to microfabricate carbon micro structures derived from photoresist based on UV lithography and pyrolysis process. The same research group reported electrochemical activity on pyrolysed photoresist very similar to those on glassy carbon [16].

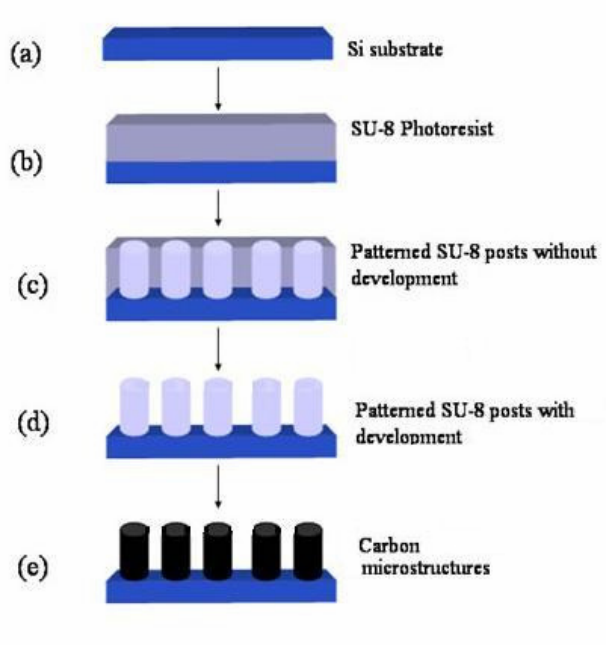


Figure 1: Typical process flow for fabricating C-MEMS structures (a) Si substrate (100); (b) SU-8 film of 100µm thickness was spin-coated; (c) patterning the photoresist by UV exposure; (d) patterning structures by EB writer, and then development; (e) finally pyrolysing the photoresist patterns to convert them to carbon structures.

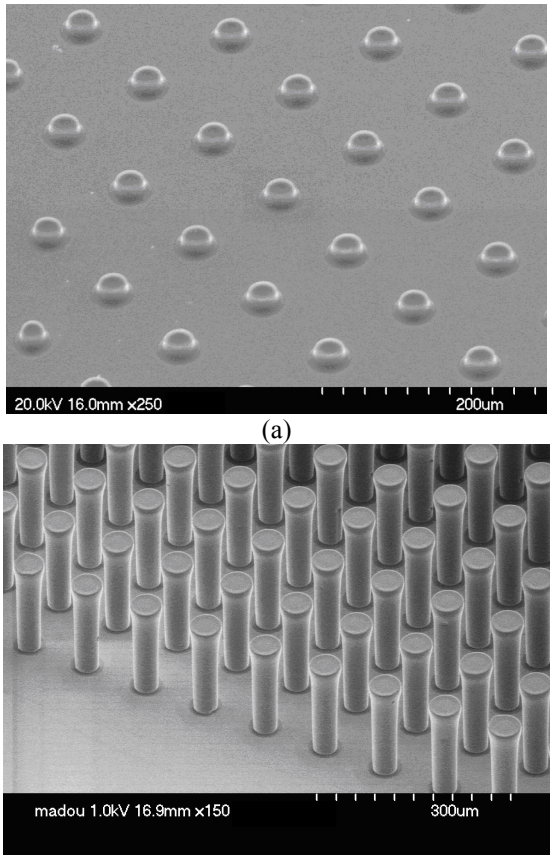
Glucose oxidize (GOx) is immobilized in the process of electrochemical polymerization of polypyrrole (PPy). Polymer film 5µm thick is deposited on the carbon structure (1cm^2 in area), utilizing potentiostatic mode (0.65V v.s. Ag/AgCl reference electrode, gold plate as counter electrode) of a PC4/750 potentiostat (Gamry Instruments). Dodecylbenzenesulfonate (DBS^-) was used as dopant species during the polymerization process. The electropolymerization solution was 0.1 M potassium phosphate buffer (pH 7.0 at 25ºC) consisting of 0.1M pyrrole, 0.1M NaDBS and 100Uml^{-1} GOx. After electropolymerization, the derived glucose sensor was washed several times with distilled water to remove any loosely bound enzyme and pyrrole monomer, and then stored in 0.1 M potassium phosphate buffer solution (pH 7.0).

To demonstrate the redox properties of the PPy/ GOx glucose sensor, cyclic voltammogram (CV) experiment was carried out in glucose solution (PBS) Glucose sensing test was then carried out by the same potentiostat equipment (Ag/AgCl as reference electrode, gold plate as counter electrode). The glucose stock solution (1 M) was stored in the refrigerator and diluted when necessary to give the

required standard concentration with a phosphate buffer solution (pH 7.0 at 25ºC). The response of the PPy/GOx glucose senor to glucose with various concentrations was examined in 25ml of phosphate buffer solution at a constant potential (0.7V v.s. Ag/AgCl). After the background current became constant, specific amounts of 1M glucose were added to the solution to produce glucose concentration ranging from 0.5mM to 20mM. The sequential current changes corresponding to the increasing glucose concentrations were recorded by the same potentiostat.

3 RESULTS AND DISCUSSION

Typical SEM pictures of carbon posts with different heights (20μm and 140μm) fabricated by this method are shown in Figure 2. These carbon microstructures are about 30μm in diameter and adjacent posts are 70μm apart.



(a)

(b)

Figure 2: SEM pictures of Carbon posts electrode array on carbon substrate (a) post height 20μm (b) post height 140μm.

Cyclic Voltammetry of PPy/GOx post in glucose solution with glucose concentration of 1mM is shown in Figure 3. The scan rate is 30mV/s, the scan range is from -0.2V to 1V. range from -0.2V ~ 1V. An oxidation peak, corresponding to the reaction $H_2O_2 \rightarrow O_2 + 2H^+ + 2e$, existed at approximately 0.7V, which we choose as the static working potential in our experiments. The static working potential, i.e. 0.7V, was in good agreement with literatures and independent of the electrode material, fabrication technology and surface modification.[17]

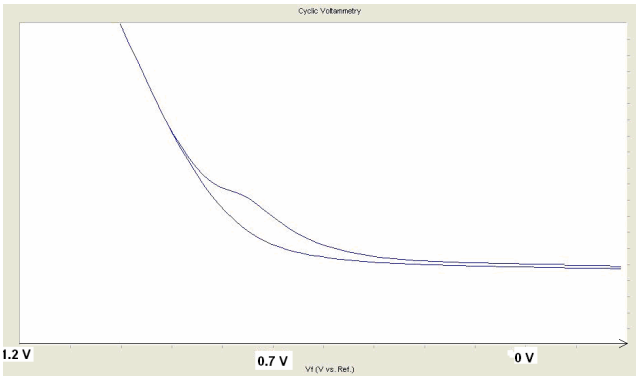
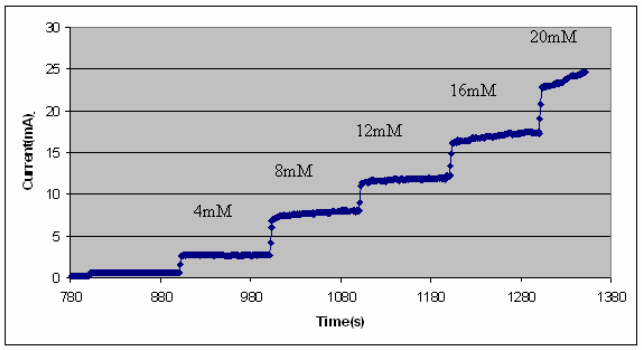
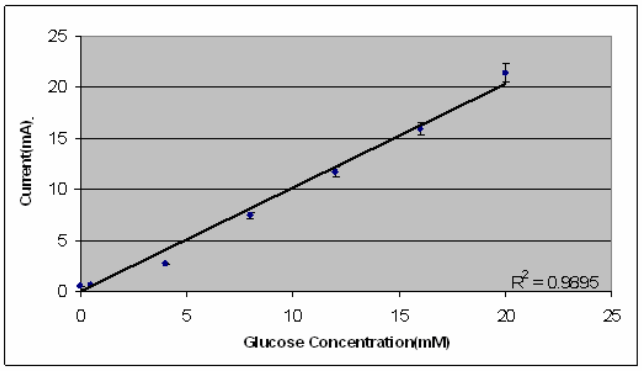


Figure 3: Cyclic Voltammetry of PPy/GOx post in glucose solution with glucose concentration of 1mM in PBS(pH 7 at 25ºC). The scan rate is 30mV/s, the scan range is from -0.2V to 1V.



(a)



(b)

Figure 4: (a)A typical sensing curve of the PPy/GOx on carbon film. (b) Response of PPy/GOx on carbon film to glucose concentration.

The sensing response of the PPy/GOx on carbon film to glucose solution with various concentrations is demonstrated in Figure 4. From Figure 4(a) we estimated

the response time of the sensor to glucose is around 20s, which is in agreement with the response of conventional glucose sensors.[18] The linear fitting line in Figure 4 (b) illustrates a linear range of 0.5mM ~ 20mM, the sensitivity of the glucose sensor can be read from the slope of the fitting line.

Post height (μm)	0 (film)	20	85	140
Sensitivity (mA/mM)	1.02	1.28	1.74	2.02

Table 1: Sensitivities of glucose sensors on carbon posts of different heights.

Sensitivities of different PPy/GOx post glucose sensors are summaried in Table 1. The result demonstrates larger sensitivity with higher posts. Sensitivity of 140μm post is about two times that of carbon film. This may result from more reaction sites on the surface of higher posts due to their larger surface area. To further enhance performance of the glucose sensor, optimization of electrode geometry and preparation parameters of the PPy/GOx covered carbon post glucose sensor is yet to be investigated. Further work also includes miniaturization of the sensor by using smaller footprint area while achieving high linear range.

4 CONCLUSION

Design of a novel glucose sensor based on high aspect ratio carbon post electrode array has been reported in this work. Fabrication process of the high aspect ratio carbon post electrode array and immobilization of enzyme onto the electrodes through electrochemical polymerization of pyrrole are also described. Glucose sensing performance for 140μm, 85μm and 20μm high carbon post (diameter 30 μm), as well as carbon film are tested and compared. The result demonstrates larger sensitivity with higher posts, which could be explained by more reaction sites due to their larger surface area. This property provides feasibilities of developing miniaturized glucose sensor without losing signal quality. Response of the PPy/GOx post glucose sensor shows a linear range from 0.5mM to 20mM and a response time of around 20s, which offers possibilities of its practical application in monitoring blood glucose level of diabetic patients.

Acknowledgements: This work was funded by NSF DMI-0428958. The author Han Xu would like to thank Dr. Lawrence Kulinsky for the discussion.

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