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Integration of carbon nanotubes to three-dimensional C-MEMS for glucose sensors

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Abstract:
A novel design and fabrication of glucose sensors based on carbon nanotubes (CNT) integrated with carbon micro-electromechanical system (C-MEMS) were reported in this work. Photolithography of CNT mixed SU-8 photoresist and the self-assembly of CNTs during the developing process were followed by the controlled pyrolysis process to form CNT integrated three-dimensional C-MEMS electrode array. Immobilization of enzyme onto these electrodes was carried out through co-deposition of glucose oxidase (GOx) and electrochemically polymerized polypyrrole (PPy) for glucose sensor. Sensing performance with different electrodeposition time and various glucose concentration were tested and compared. The results revealed that the C-MEMS/CNT based glucose sensor increased approximately two order of magnitude in sensitivity compared with blank C-MEMS one, demonstrating linear range for glucose concentrations from 1 mM to 15 mM with fast response.

Key words: glucose sensors, CNT, C-MEMS, integrated

Introduction

Diabetes mellitus is a serious metabolic diseases caused by insulin deficiency affecting more than 200 million people in the world [1,2]. Since the introduction of intensified insulin therapy, patients are recommended to self-monitor their blood glucose level at least four times daily to assess whether glycemic targets are being achieved [3]. The increasing trend in the treatment of diabetes impell researchers to develop novel glucose sensors with high sensitivity, good reliability, and excellent selectivity [4–6].

Biosensors employing carbon nanotube electrodes have been demonstrated to have a faster response time and higher sensitivity than those employing traditional platinum electrodes [7-9]. The integration of CNTs into biosensing electrodes has been challenging [10]. Coincidentally, our previous work provided a facile approach to fabricate C-MEMS/CNT 3-D structure, which demonstrated improved electrical performance compared with blank C-MEMS [11]. In the past few years, C-MEMS technique has been successfully developed with the advantages of flexible design, high-resolution, low-cost and good repeatability, and proved to be effective in the application of electrode in electronic and biological devices [12,13]. Concerning the good bio-compatibility of carbon material, the C-MEMS electrode offers advantages for life-science related applications.

On the other hand, high aspect-ratio nanomaterial carbon nanotubes (CNTs) have attracted extensive attention due to its unique properties such as significant mechanical strength, high surface area, excellent electrical conductivity and high chemical stability [14]. Studies on CNT-integrated electrode revealed that CNTs can speed up the interfacial electron transfer [15,16]. Nanotubes have also enabled high-sensitivity detection of charge, mass and spin, and have steadily approached the quantum limit of mechanical motion [17]. Biological entities such as proteins, enzymes and bacteria can be easily immobilized either in the hollow cavity or on the surface of CNTs, and then the functional CNTs can be applied in high-sensitive biosensors to selectively detect of biological and chemical species [18,19]. The outstanding electronic properties of the CNTs, coupled with the specific recognition capability of the immobilized species on CNTs make CNTs an ideal biosensor component [20–22]. The integration of CNTs to C-MEMS structures would not only greatly increase the surface area, electrical conductivity, but also allow for direct electron transfer and easy enzyme immobilization when applied into biosensors.

Thus, we propose the novel approach to apply the C-MEMS/CNT integrated structure to bio-electrode of glucose sensors in order to improving the sensing performance of biosensors.

Experiment

The experimental procedure to prepare CNT/C-MEMS bio-electrode was presented in Fig. 1. A carbon film was applied as current collector, which was realized by spinning coating a nagetive photoresist, ENPI, on a silicon substrate at 1000 rpm for 10 s and then at 2000 rpm for 30s, followed by a 3 min soft bake at 120 ºC. A glassy carbon film substrate of ~2 µm thickness was obtained by a pyrolysis of the photoresist film at 1000 ºC (heating rate 2 ºC /min) for 60 min in forming gas (5% H_2 in N_2) environment. The procedures for the preparation of the CNT/C-MEMS composite have been reported previously [9]. In this work, we have chosen single-walled carbon nanotubes (SWCNT) to construct the fractal composite. Furthermore, in order to take full advantage of the self-assembly effect of CNT, development was performed using SWCNT mixed SU-8 developer.

The GOx enzyme for amperometric glucose sensors was immobilized onto the carbon post-microarrays in the process of electrochemical polymerization of PPy. Dodecylbenzenesulfonate (DBS−) was used as dopant species during the polymerization process. The electrodeposition process was performed in potentiostatic mode (0.7 V) by an electrochemical workstation (CS310, Corr Test Instrument Co., Ltd) with three-electrode arrangement (a platinum wire counter electrode, a Ag/AgCl reference electrode and a carbon working electrode). The solution for electrochemical polymerization consisted of 0.1M potassium phosphate buffer (pH 7.0 at 25 ◦C), 0.1M pyrrole, 0.1M NaDBS, and 100Uml^{-1} GOx. After electrochemical polymerization, the derived glucose sensor was washed several times with distilled water to remove any loosely bound enzyme and pyrrole monomers, and then stored in 0.1M potassium phosphate buffer solution (pH 7.0 at 25 ◦C).

The structure of the products were characterized using a scanning electron microscope (SEM, JEOL JSM-5510LV) operated at 20 keV. Glucose sensing test was implemented using the same potentiostat equipment.

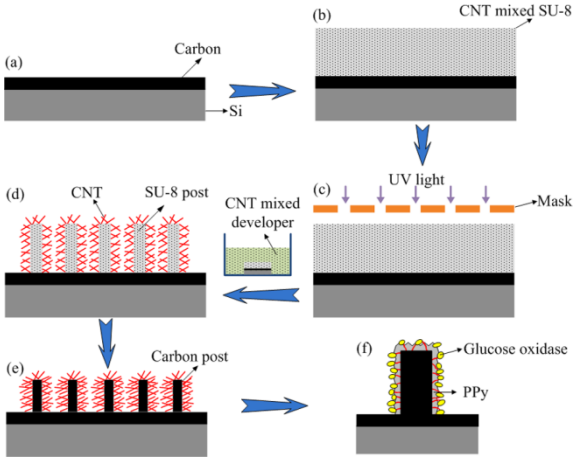

Fig. 1. Process flow for fabrication of CNT/C-MEMS bio-electrode (a) preparing the carbon current collector on silicon substrate; (b) spinning-coating of SWCNT mixed SU-8 photoresist over carbon film; (c) patterning the photoresist by UV exposure; (d) developing the SU-8 structures in SWCNT mixed developer; (e) pyrolyzing the SU-8 patterns to convert them into carbon structures; (f) electrodeposition of PPy/GO_x on CNT/C-MEMS electrode.

Result and discussion

Fig. 2 shows the typical SEM images of blank C-MEMS [Fig. 2 (a) and (b)] and C-MEMS/ CNT composites [Fig. 2 (c) and (d)]. Dense SWCNTs coverage is observed on the carbon microelectrode surface, especially on sidewalls. Typical SEM photo of the C-MEMS after the electrochemical deposition of PPy/GO_x is shown in Fig. 3. Glucose sensing test was implemented using Ag/AgCl as reference electrode and platinum wire as counter electrode. Both C-MEMS and CNT/C-MEMS electrodes with electrochemical deposition for 120 s and 240 s were measured, shown in Fig. 4, demonstrating that the C-MEMS/CNT based electrode results in approximately two order of magnitude increased sensitivity compared with blank C-MEMS one. This could be explained by the presence of more reaction sites on the larger reactive surface area of the C-MEMS/CNT based glucose sensor. Furthermore, the introduction of CNT into biosensor results in the direct electron connection of GOx enzyme with electrode, and thus speed up the electron transfering. The least-square linear regression analysis of the calibration characteristics in Fig. 4(e) and (f), gives a correlation coefficient more than 0.9, indicating that the amperometric response signal of the glucose sensors is in the linear range for glucose concentrations from 1 mM to 15 mM.

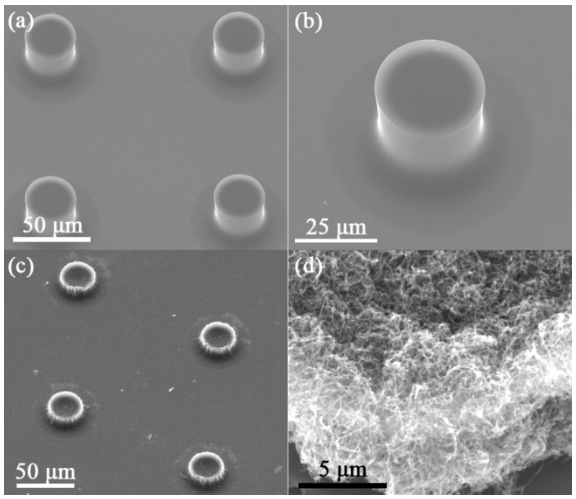

Fig. 2. SEM images of (a) and (b) blank C-MEMS; (c) and (d) CNT/C-MEMS composite. (a) and (c) are the low magnification view, while (b) and (d) are higher magnification image of the corresponding posts.

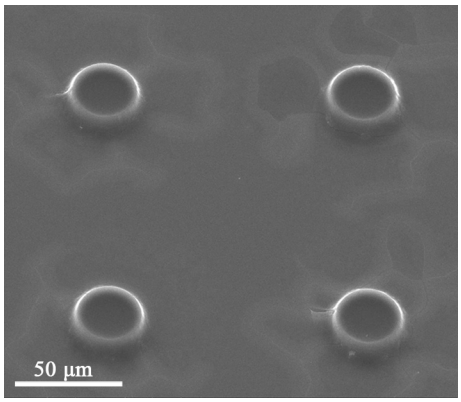

Fig. 3. SEM image of PPy/GO_x modified C-MEMS electrode.

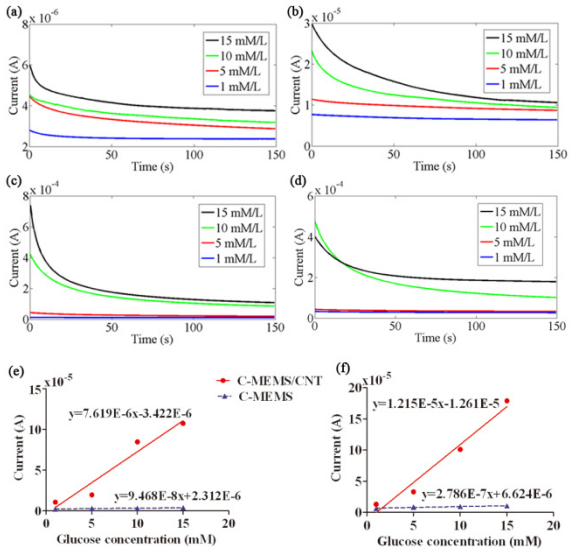

Fig. 4. Typical sensing curves of bioelectrode in glucose solution with concentration of 1, 5, 10, 15 mM/L (a) C-MEMS electrode with the electrodeposition time of 120 s; (b) C-MEMS electrode with the electrodeposition time of 240 s; (c) C-MEMS/CNT electrode with the electrodeposition time of 120 s; (d) C-MEMS/CNT electrode with the electrodeposition time of 240 s. (e) Calibration plot for C-MEMS and C-MEMS/CNT with the correlation coefficient of 0.9847 and 0.9382, respectively, and the electrodeposition time of 240 s; (f) Calibration plot for C-MEMS and C-MEMS/CNT with the correlation coefficient of 0.9168 and 0.9673, respectively and the electrodeposition time of 240 s.

Conclusion

An amperometric glucose biosensor was developed by co-immobilizetion GOx in PPy on CNT integrated C-MEMS electrode. Amperometric responses of this integrated electrode for glucose detection demonstrate a linear range from 1 mM to 15 mM, which offers possibility of its practical application in monitoring blood glucose level of diabetic patients. The experimental results show that the sensitivity of the C-MEMS/CNT bioelectrode is around two order of magnitude higher than that of C-MEMS, which could be explained by the presence of more reaction sites on the larger reactive surface area of the C-MEMS/CNT based glucose sensor, and also the direct electron connection of GOx enzyme with electrode. Therefore, it's feasible to increase the sensitivity of the miniaturized glucose sensors without losing signal quality by integrating CNT with C-MEMS to construct 3-D fractal electrode.

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