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Human-Urine Diabetes Assay and *In Vivo* Rat Bladder Assay Using a Fluorine-Doped Carbon Nanotube Catheter Sensor

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Abstract—The creation of a novel biosensor consisting of a fluorine-doped carbon nanotube (FCN) was explored for use in cyclic voltammetric (CV) and square-wave stripping voltammetric (SW) glucose assay. In the experiment that was carried out in this study, analytical optimum conditions were attained at the low detection limit (S/N3) of 0.6 μg/L (3.3×10^{-9} M). In the 0.1 mg/L spike, the relative standard deviation of 0.607 ($n = 15$) was obtained. This was used for the diagnosis of the urine of patients with diabetes. Moreover, the catheter-type electrode (CE) can be inserted into a rat bladder through the rat's organs. Thus, it can be connected with an electrochemical analyzer that can be fitted with an interface for the real-time *in vivo* analysis of metabolic glucose. The developed system can be used for organ treatment, biological analysis, and *in vivo* control.

Keywords—Glucose, Voltammetry, Assay, Diabetic, Fluorine, Nanotube.

INTRODUCTION

In *in vivo* and *in vitro* diagnosis, glucose assay is important for obtaining metabolic information regarding pathologies, such as diabetes mellitus,[6] cardiovascular disease,[19] Alzheimer's disease,[20] and muscle fuel energy.[16,24] Moreover, glucose is related to the bladder's micturition-prolonged pressures[5,10] and bladder tumor.[2] Thus, nontreated *in vivo* assay and a low analytical detection limit (DL) are demanded for medical treatment. Various diagnostic detection methods were recently investigated to achieve this end, such as the capillary electrophoresis UV detection method (DL: 4.01 mg/L),[4] high-performance liquid chromatography with refractive index detection (DL: 0.13 mg/mL),[3] the capillary zone electrophoresis method (DL: 1.0×10^{-6} M),[23] and the flow injection amperometric method (DL: 0.06 mM).[15] All these methods, however, involve the use of complicated and expensive instruments. Simplified electrochemical voltammetric methods that are inexpensive and fast were thus developed in this study.[12,13,25] Nonetheless, these methods can be used only under laboratory conditions, have high detection limits, and cannot be used in *in vivo* direct assay in organs. Some examples of such methods are the bienzyme carbon paste electrode method (DL: 1 mM),[9] the bienzymatic biosensor method (DL: 0.07 mM),[8] the organically modified sol–gel glass sensor method (DL: <0.1 mM),[18] enzyme inhibition assay with the amperometric glucose biosensor (DL: 0.2 ppb),[1] the flow injection analysis (FIA) biosensor method (DL: 0.1 mM),[14] and the Prussian blue modified glassy carbon biosensor method (DL: 1.0×10^{-6} M).[22] A more advanced and effective analytical technique is urgently needed, though. Thus, in this study, the performance of a fluorine-doped carbon nanotube (FCN) sensor was investigated. Fluorine has a large electron capacity[11] and a hydrophilic or hydrophobic reactivity.[21] These redox properties are useful in –F–C– binding with a carbon nanotube, which has a catalytic effect[7] and electron capabilities.[12] The covalent bonds of $x\mathrm{C} + 1/2\mathrm{F}_2 + y\mathrm{HF} \rightarrow \mathrm{C}x\mathrm{HF}_2(\mathrm{HF})(y-1) \rightarrow \mathrm{C}x\mathrm{F}(\mathrm{HF})y$[17] produce the hydrogen bonds of –C*x*–(H–F)*n*–. The carbon nanotube can be reactive with glucose as the –[C*x*–(H–F)*n*]*n* + glucose(+) ↔ glucose(−) ion. In this study, the redox peak current was attained at a low detection limit, showing that the carbon nanotube sensor can be used for *in vivo* diagnosis.

EXPERIMENTAL PROCEDURE

Device Fabrication

Electrochemical instruments were used with the new system of *Bioelectronics-1*, which was first constructed at the authors' institute. The new version

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is a computerized handheld voltammetric sensor with a ±2.4 V potential range, a 2 mA current range, a 10-pA measuring current, and a 5" × 4" × 1" instrument size, and which uses a rechargeable battery or external power and has a USB port interface with a PC. The instrument's size is similar to that of a typical cellular phone and can be used for the bioassay and sensor techniques for individual and laboratory applications.

Electrode Preparation

The electrode materials of carbon nanotubes were used (by catalytic CVD; outside diameter: 1540 nm; length: 30–50 nm; Nanotech Co., Ltd., Choongnam 330-816, South Korea). The paste electrode was prepared with a weight ratio of 4:4:2 W, the nanotube, HF, and mineral oil. Thus, the FCN electrode was prepared with 40% carbon nanotube, 40% HF solution (50% Fluka fluoroboric acid in water, BF_4H; M_W: 87.81), and 20% reagent-grade mineral oil (New Jersey, USA 1-800-01, Acro). The FCN electrode systems were immersed in a solution of 10-mL 0.1 M H_3PO_4 electrolyte. Fluorine immobilization was performed with a 10-cycle scan, with a 1.0 V initial potential, −1.0 V switching potential, and 0.5 V/s scan rate. Under these conditions, the FCN was stabilized and then used for the optimization. The fluorine-doped carbon nanotube catheter-type electrode (FCNCE) was prepared by inserting the mixed paste (working electrode) into the 3-mm-diameter and 100-mm-long catheter capillary, then attaching to its tip a 0.2-mm-diameter chloride-coated silver wire (as an Ag/AgCl/KCl reference electrode) and a 0.2-mm-diameter platinum wire (as a counter electrode) and connecting these three electrodes to the electrochemical system.

In Vivo Implantation

Healthy male and female rats (data: Wistar/ST (1998); age: 10 w; weight: 220–250 g) were obtained from the authors' institute. The rats were anesthetized using a piece of cotton wet with 10 mL ethyl ether and were fixed on a 200 × 100 × 10-mm^3 polyethylene plate using a binder tape. The FCN was coated with petroleum jelly. The FCNCE was very small, which made it easy to insert it into a rat bladder or any other organ, for that matter. All the other reagents that were used were of reagent grade (Sigma) and were used with triple-distilled water. An experiment on electrode cleaning was found unnecessary as each measurement could be performed in an open circuit, at room temperature, without oxygen removal.

RESULTS AND DISCUSSION

Cyclic Voltammetry and Electrode Comparison

First, the redox peak potential and the glucose variation were examined using cyclic scanning with FCN, under optimum conditions. Figure 1a shows the 8-point addition. In the blank solution, a clear curve appeared, then the concentration was continuously spiked. At a positive scan, a 0.15 V peak potential was obtained, after which a negative reaction appeared at a small peak in the same voltage, where linear working curves were obtained for the 5–40 mg/L ranges.

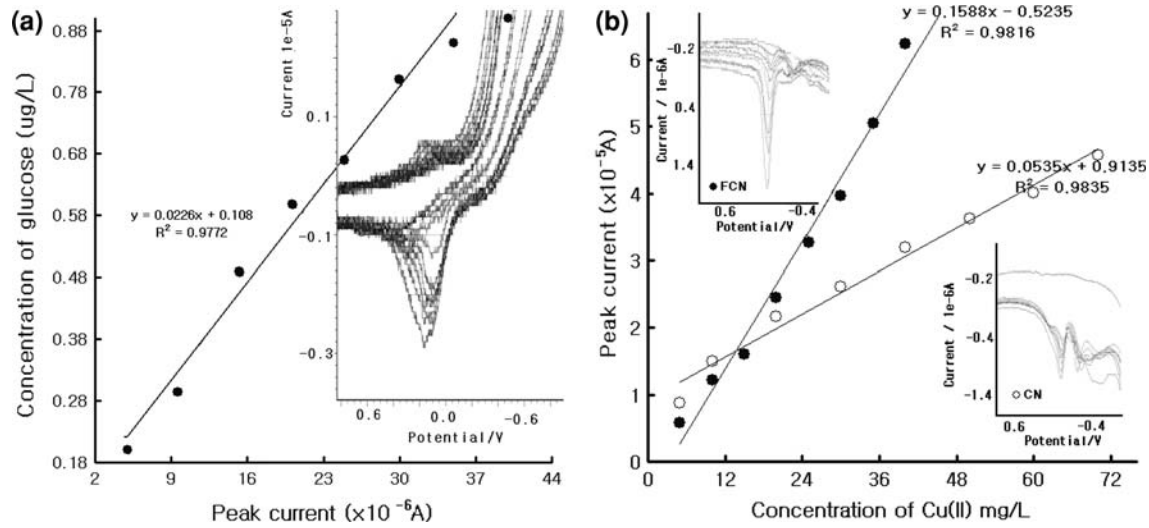

FIGURE 1. (a) CV concentration effects using FCN with 0–40 mg/L glucose variation, and with optimum conditions of −1.0 V initial potential, 1.0 V switching potential, and 0.1 V/s scan rate. (b) SW glucose variation for the FCN (0–40 mg/L spike) and the CN electrode (0–70 mg/L spike) in a 0.1 M H_3PO_4 electrolyte solution. Other parameters were used under optimum conditions.

In these equations, a precision of RSD = 0.997 and a slope ratio of $\Delta x/\Delta y = 0.0226$ were obtained. This is useful for pharmaceutical or other forms of glucose level analysis. Under these conditions, the electrode sensitivities were compared using a nontreated carbon nanotube (CN) electrode and an FCN electrode. The experimental results are shown in Fig. 1b. Different working ranges appeared, the FCN (0–40 mg/L) was short, the CN (0–70 mg/L) was long and had a poor slope, the slope ratio of the FCN electrode ($\Delta x/\Delta y = 0.15880$) was more sensitive than that of the CN electrode ($\Delta x/\Delta y = 0.0535$), and the FCN peak width was very sharp. Moreover, at the 40 mg/L spike, the FCN peak height (6.41×10^{-7} A) increased two-fold, followed by the CN peak (3.471×10^{-7} A). Moreover, both electrodes responded at identical peak potentials, and no effects of the interference of fluorine were observed. Under these conditions, better sensitive working ranges were examined using SW optimization.

Stripping Optimizations of the FCN Electrode

For the optimum conditions, the SW parameters were determined using FCN. Figure 2a shows the SW amplitude variations for 8 points in the 30 mg/L glucose spike. At 0.45 V, the maximum current was obtained at a 21.07×10^{-6} A peak height and with a broad and insensitive peak width. Thus, the optimum conditions that were chosen for 0.35 V were sensitive, for which a big current was also used. Under these conditions, the SW increment potentials were examined for the variation from 0.005 to 0.04 V with 8 points. Figure 2b shows the raw voltammograms. The peak current continuously increased, and the maximum current of 3.654×10^{-6} A was obtained at 0.04 V. The peak width was narrow, and the 0.35 V amplitude and 0.04 V increment potential were fixed. Under these conditions, the SW frequency was examined for 8-point variations. Figure 2c shows the raw voltammograms. At 5 Hz, a sharp increase in the peak height (5×10^{-6} A) appeared, and the peak width was also sharp. Then other optimum influence parameters were obtained, such as 1.0 V accumulation potential, 1.0 V final potential, 5.92 pH strength, 150 s accumulation time, 0.35 V amplitude, 0.04 V increment potential, and 5 Hz frequency. The analytical working ranges were examined using these parameters.

Working Ranges, Statistics, and Application

Under the optimum conditions, the analytical working ranges were examined using FCN. Figure 3a shows the results of the microgram variation. The width of the glucose peak narrowed at 0.1 V, with a slope sensitivity of $\Delta x/\Delta y = 0.0414$, an intercept of 3.2104, and a precision of $R^2 = 0.9968$. The results had very low ranges, which shows that DL is useful for the assay of diabetes and cardiovascular risk. The applications were performed on a healthy patient's urine. Figure 3b shows the standard addition method of urine from a healthy male (age: 20 years; weight: 65 kg). The first curve represents the blank electrolyte solution, after which the human urine was spiked at 0.5 mL and obtained a small peak. Thus, the 30, 60, and 90 μg/L glucose standards were continuously spiked, and a sensitivity of $\Delta x/\Delta y = 0.0855$, an intercept of 6.7907, a precision of $R^2 = 0.9817$, and a calculated value of 6.619 ± 0.06 μg/mL ($n = 3$) were obtained. Then the urine of a female patient with diabetes (age: 46 years; weight: 67 kg) was examined, and 26.79 ± 0.16 μg/mL ($n = 3$) was obtained, which showed very high patient peaks that can be useful for

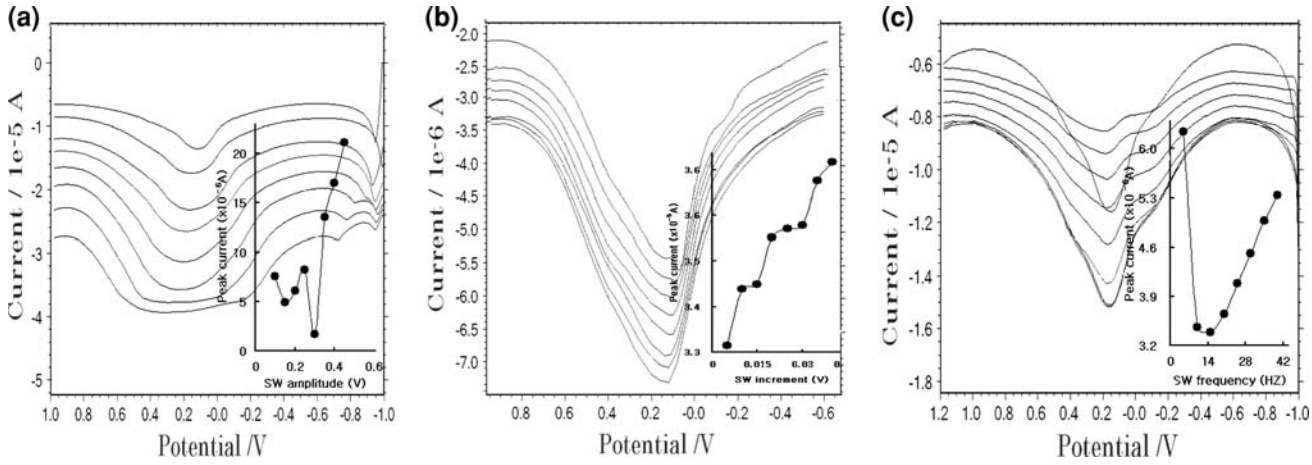

FIGURE 2. (a) SW amplitude variations from 0.1 to 0.45 V. (b) SW increment potentials from the 0.005 to 0.04 V variations. (c) Various SW frequencies from 5 to 40 Hz, using 30 mg/L glucose spiking in a 0.1 M H_3PO_4 electrolyte solution. Other parameters were used under optimum conditions.

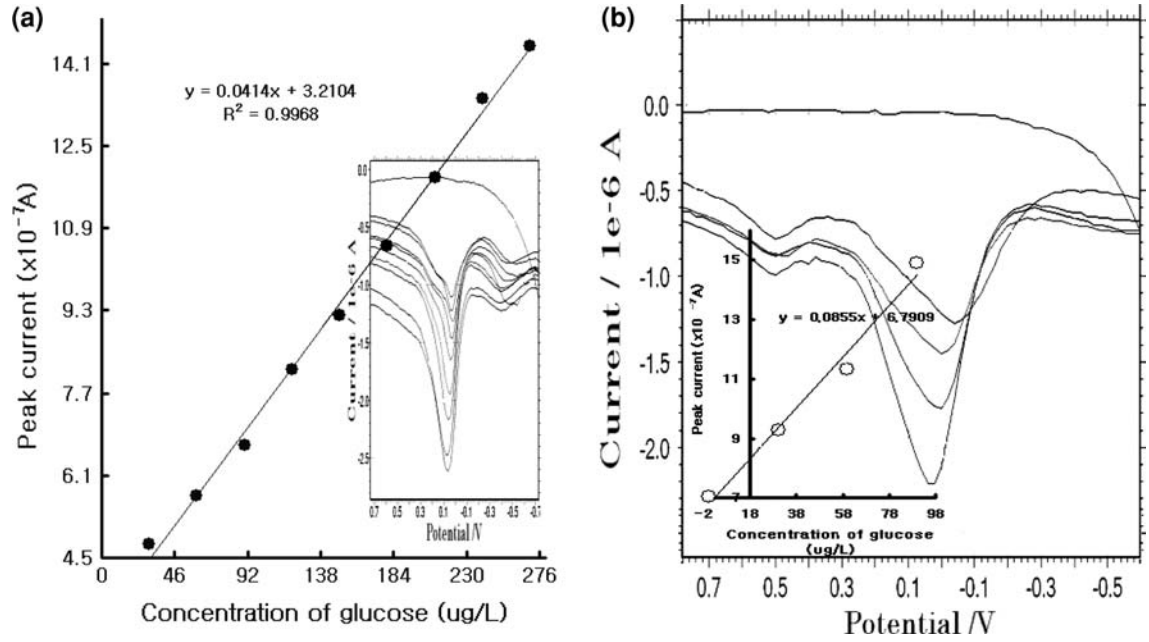

FIGURE 3. (a) SW anodic variations for the 0, 30, 60, 90, 120, 150, 180, 210, 249, and 270 μg/L spikes in a 0.1 M $NH_4H_2PO_4$ solution, with −1.0 V accumulation potential, 1.0 V final potential, 5.92 pH, 150 s accumulation time, 0.35 V amplitude, 0.04 V increment, and 5 Hz frequency, whereas other parameters were set at the optimum conditions. (b) The standard addition method, where the first curve is a blank electrolyte solution, followed by a 0.5 mL healthy-urine spike then 30, 60, and 90 μg/L standard glucose spikes under the same optimum conditions as in (a).

diabetes detection. The voltammograms are not shown here.

FCNCE In Vivo Application on a Rat Bladder

For *in vivo* application, a macro-type FCNCE was prepared. Figure 4a shows the flexible fabrication of the FCNCE. The three combined electrode systems can be inserted into animal or human organ systems. In addition, the electrode diameter is very narrow, making the device handy. Under anesthetized conditions, the petroleum-coated FCNCE was simply inserted into the rat bladder. Figure 4b shows X-ray photo films of the cable that was implanted in the fixed body. The *in vivo* SW conditions were different from those of the electrolyte cell systems; as such, the raw bladder conditions were reorganized. Moreover, the −1.0 V initial potential, 0.04 V increment potential, 0.25 V amplitude, and 10 Hz frequency parameters were changed. Under these conditions, SW voltammograms of a 0.68×10^{-6} A peak current were obtained for the G peak. Figure 4c shows the results of the SW peak potential at 0.2 V, which was very sensitive and indicates that the developed methods can be applied to *in vivo* diagnosis. Figure 4d shows the CV voltammograms under identical conditions. The same peak potential appeared for the 1.45×10^{-6} A peak current using the −1.0 V initial potential, 1.0 V switching potential, and 0.1 V scan rate. The CV voltammograms can also be used in *in vivo* applications. Using FCNCE, the statistic ability was examined with the 20 mg/L glucose spike, and the stripping was repeated with the optimum parameters. The first seven points were gradually increased from the 6.24×10^{-5} to the 10.02×10^{-5} A peak current, but the other 10 points were linearly oscillated from 9.652×10^{-5} to 12.71×10^{-5} A, then stabilized. At each instance, the working surface was polished using weighing papers with Al_2O_3 powder, and the FCNCE stability was obtained within a ±5% margin of error at each voltammogram. The flash FCNCE was used for 1 or 2 months.

CONCLUSION

The efficiency of the first fluorine-doped carbon nanotube biosensor was examined through the voltammetric assay of glucose. Under the optimized conditions, a 150-s accumulation time was used and attained within the working ranges of 30–270 μg/L SW and 5–40 mg/L CV. The lowest DL that was obtained was 0.6 μg/L (3.3×10^{-9} M) SW (S/N = 3) and was more sensitive than that in the previous method of separation[3,4,15,23] and in the electrochemical[8,9,14,18,22] methods. The FCNCE macrosensor was first applied to the *in vivo* diagnosis of a rat bladder and to the urine of patients with diabetes. The results of this study

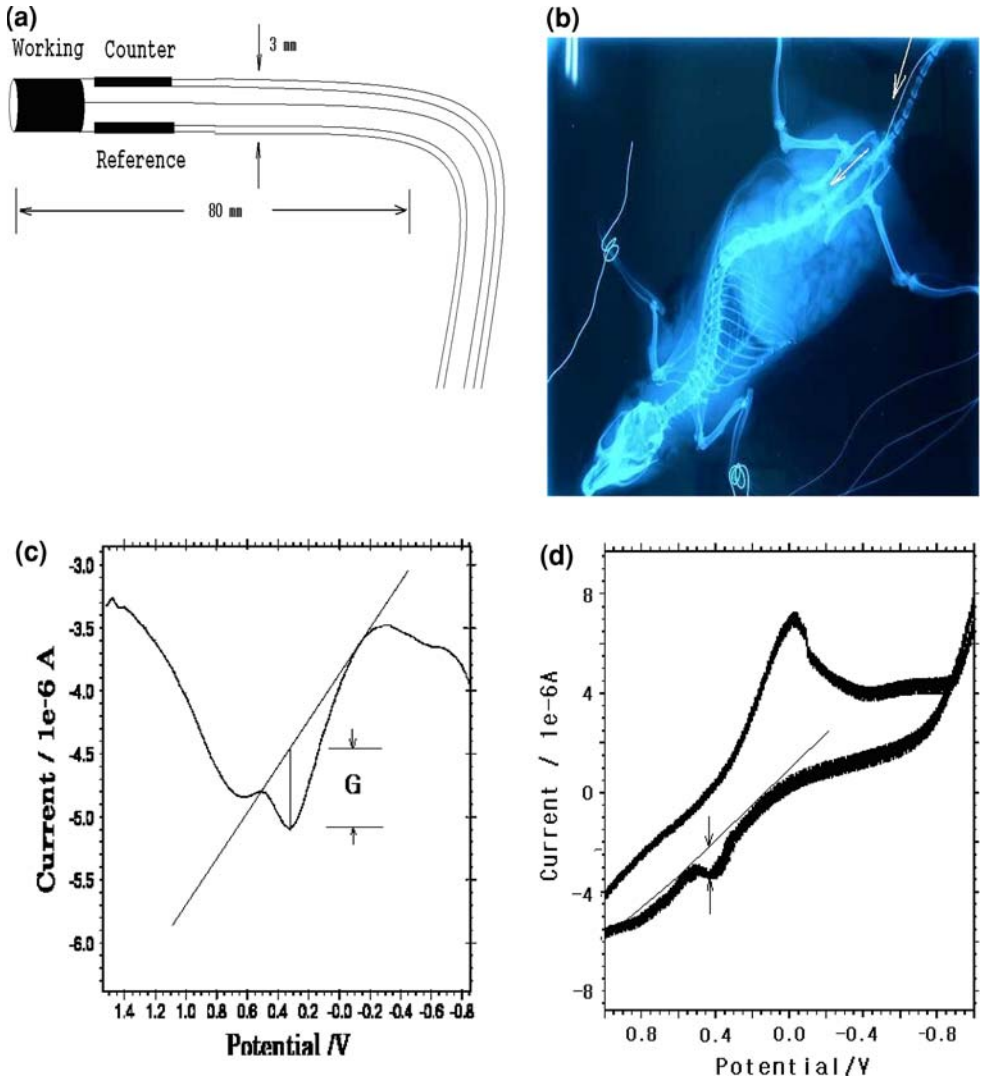

FIGURE 4. (a) Fabrications of the implantable FCNCE sensor. (b) X-ray photo film for the implanted FCNCE in the rat bladder. (c) Glucose peak current with the optimum SW under the same conditions as in (b). (d) Cyclic voltammogram under the same conditions as in (b).

indicate that the developed method can be used for the real-time biological *in vivo* assays of organs and of other materials, pharmaceuticals, foods, and drugs.

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