An amperometric non-enzymatic glucose sensor by electrodeposition copper nanocubes onto vertically well-aligned multi-walled carbon nanotube arrays

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A non-enzymatic glucose (Glc) sensor was developed by potentiostatically electrodedepositing metallic Cu nanocubes from a precursor solution onto vertically well-aligned multi-walled carbon nanotube arrays (MWCNTs). The electrochemical characteristics of the sensor were studied by electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV). The sensor shows significantly higher electrocatalytic activity to the oxidation of Glc in 0.1 M NaOH alkaline solution after modification of Cu nanocubes than before. The sensor response is rapid (<1 s) and highly sensitive (1096 µA mM⁻¹ cm⁻²) with a wide linear range (up to 7.5 mM) and low detection limit (1.0 µM at signal/noise ratio (S/N) = 3); it also exhibits high stability and specificity to Glc and performs very well in detecting Glc concentration in human blood serum.

1. Introduction

The pursuit of a rapid, simple, inexpensive, and reliable Glc sensing is on going for various applications including for clinical application of blood Glc sensing, ecological application of industrial waste water treatment and food application of dietary and obesity control. Glc enzyme electrodes based on glucose oxidase (GOx), which oxidizes Glc into gluconic acid in the presence of oxygen, and cross-linking and entrapment in a complex matrix and poor specificity to Glc.

Cu is one of the widely investigated metal catalysts and its electrodeposition has been studied on various carbon scaffolds (Schmidt et al., 2007). The unique structural, physical, and chemical properties of nanoscaled materials are closely related to both their size and shape (Zhang et al., 2008). Cubic Pt particles are said to exhibit higher catalytic activity with more effective active sites, than spherical particles (Yamada, 2009). Thus, controlling the nanomaterial morphology is an effective way to impart certain unique properties to nanostructured materials. Synthesis of various nanostructures has been achieved using different techniques: cubes (Chen et al., 2007; Claussen et al., 2009), wires (Zhuang et al., 2008), platelets (Wang et al., 2008a,b), spindles (Zhang et al., 2008), rods (Ren et al., 2009) and prisms (Pal et al., 2009). For example, Zhang et al. (2008) synthesized CuO nanostructures of wires, platelets, and spindles and used them for Glc sensing by simple wet-chemistry and Kang et al. (2008) electrochemically deposited Cu nanoclusters onto a MWCNTs-modified glassy carbon (GC) electrode. However, most fabrications of nanostructures involve complicated multi-steps.

Herein, we describe a simple electrodeposition method to synthesize novel nanocomposites of Cu nanocubes and MWCNTs for Glc sensing, which has not been reported. MWCNTs arrays were grown on Ta substrate by catalytic vapor deposition (Zhang et al., 2002) and Cu nanocubes were electrochemically deposited onto the MWCNTs arrays by a simple cathodic potentiostatic technique. The Cu-modified MWCNTs electrode shows considerably higher...
electrocatalytic activity for Glc than the unmodified MWCNTs. This new nanocomposite material combines the advantages of Cu nanocubes and MWCNTs arrays (Katz and Willner, 2004) and could exhibit a synergistic effect. The Cu-MWCNTs nanocomposite electrode exhibits high sensitivity, stability, and specificity for Glc. Its response is rapid with a wide linear range, a low detection limit and good performance in detecting Glc content in blood serum. These qualities make the Cu-MWCNTs electrode a promising candidate for enzyme-free amperometric Glc sensing.

2. Experimental

2.1. Chemicals and reagents

d(+)-Glucose, L-ascorbic acid (AA), uric acid (UA), dopamine (DA), D-fructose, mannose, and lactose were purchased from Alfa Aesar. All other reagents were of analytical grade and were used as received without further purification. All solutions were freshly prepared with high-quality deionized water (resistivity > 18.4 MΩ cm⁻¹).

2.2. Instruments

XRD profiles of Cu-modified MWCNTs were obtained (XD-3A, Shimadzu) with high-intensity Cu Kα radiation (λ = 1.5406 nm). Morphologies and nanostructures of vertically-aligned MWCNTs arrays and Cu nanotubes were determined using transmission electron microscopy (TEM) (Hitachi H7650, Tokyo, Japan) and scanning electron microscopy (SEM) (Hitachi S-3700N, Tokyo, Japan) which is equipped with energy dispersive X-ray spectroscopy (EDS) to analyze surface elemental composition. Chemistry analyzer (Synchron CX9, Clinical System, Beckman, USA) was used to determine Glc content in human blood serum (kindly provided by Nanfang Hospital, Guangzhou, China) within 1 h of drawing blood to compare with results from our sensors.

Electrochemical measurements and EIS were performed on a CHI 660C electrochemical analyzer (Shanghai, China) and data were analyzed with CHI software. A conventional three-electrode configuration was used: Ag/AgCl (3 M KCl) reference electrode, Pt wire counter electrode, and a working electrode (unmodified Cu-modified MWCNTs) was previously determined to be the optimal duration to achieve the best electrocatalytic performance. All experiments were performed at room temperature (25 ± 1 °C). Solutions were pre-purged with N₂ for at least 15 min to remove O₂, while a continuous flow of N₂ was maintained during experiments. The configuration of the Cu-MWCNTs electrode is shown in Cherevko and Chung, 2010; Li et al., 2009; Scheme S1.

2.3. Preparation of the Cu-MWCNTs nanocomposite electrode

MWCNTs arrays were synthesized employing the catalytic chemical vapor deposition technique on 3 mm × 3 mm Ta foils (Zhang et al., 2002) and were used as unmodified MWCNTs electrode by connecting it to the surface of a GC electrode with a conductive silver paint (Structure Probe Inc., USA). Nail enamel (Maybelline, USA) was used to insulate edges of the electrode for further electrodeposition of Cu nanocubes. The copper nanocubes were electrochemically coated onto the MWCNTs array by potentiostatic deposition. A precursor solution of 10 mM CuCl₂ and 100 mM KCl was used as the copper source. A constant potential of −0.40 V was applied to the MWCNTs electrode for 120 s, which was previously determined to be the optimal duration to achieve the best electrocatalytic performance. All experiments were performed at 25 °C. Solutions were pre-purged with N₂ for at least 15 min to remove O₂, while a continuous flow of N₂ was maintained during experiments. The configuration of the Cu-MWCNTs electrode is shown in Cherevko and Chung, 2010; Li et al., 2009; Scheme S1.

3. Results and discussion

3.1. Structural characterization

A typical XRD profile of as-prepared Cu-MWCNTs is shown in Fig. 1A. The peaks labeled with * indicate reflections from Ta substrate (JCPDS 04-788) and those with # at 2θ of around 43° and 95° were very close to the profiles of metallic Cu (JCPDS 04-0836). No additional peaks of Cu or other impurities or oxidation of Cu₂O/CuO were observed. The obvious peak observed at 2θ = 22.4° was attributed to graphite carbon (002) (JCPDS 75-1261). Elemental compositions of Cu-MWCNTs were analyzed by EDS (Fig. 1B). Ta, O and Co elements were observed for unmodified MWCNTs arrays. The peak of Ta was from the substrate and the small amount of Co originated from the catalyst used during MWCNTs synthesis (Chen et al., 2008). In comparison, Cu was only observed in the spectrum of Cu-MWCNTs, suggesting Cu was successfully electrodeposited onto the MWCNTs.

Fig. 1C shows the TEM images of MWCNTs and Cu-MWCNTs. Before modification (Fig. 1C, inset), the nanotubes were smooth, with the tubular diameter of 110.8 ± 5.5 nm (six measurements), consistent with the range of 80–120 nm reported in the literature (Ye et al., 2003). After electrodeposition, the tip and sidewalls of MWCNTs were embellished with solid Cu nanocubes, giving them a high aspect ratio and large surface area. The morphology of the Cu deposits is clearly cubical with fairly uniform edge dimensions from 20 nm to 70 nm. Larger and more numerous nanocubes are present at the MWCNTs tip than at other locations, owing to the high static attraction at the tip during electrodeposition.

The morphology and structure of MWCNTs and Cu-MWCNTs were further investigated with SEM. The nanotubes were found to be vertically well-aligned with part of the nanotubes peeled off for easy observation (Fig. 1D). The surface of the unmodified MWCNTs is fairly clean and smooth (Fig. 1D, inset), whereas that of Cu-MWCNTs becomes rough with cubical Cu particles attached to the sidewalls and tips (Fig. 1E). The majority of Cu particles have a uniform cubic structure. Fig. 1E(inset) confirms the attachment of cubical Cu nanoparticles on to MWCNTs.

Though the detailed mechanism of electrochemically forming the Cu nanocubes is unclear, anion species present in the solution and the MWCNTs arrays are believed to play an important role. Anions are generally absorbed onto a crystal surface and can affect the growth rates between facets of nanoparticles (Yamada, 2009), and the additive effect of a salt can achieve cubic selectivity of up to 84% (Yamada et al., 2005). In our system, Cl⁻ present in the Cu plating solution can form aqueous Cu(1)–Cl⁻ complexes and induce strong Cl⁻–Cu surface adsorption interactions to affect the kinetics of Cu electrodeposition (Chassagne and Wiart, 1984). During nucleation under a low cathodic overpotential, Cu surface is covered by chloride-containing anion species (Magnusen, 2002) and can therefore hinder the electrodeposition. The anisotropy of Cu electrodeposits can be ascribed to the Cu(1)–Cl⁻ complexes interacting with different copper surface lattice sites (Yamada, 2009) and can determine the rate and morphology (Wu and Barkey., 2000). Furthermore, the interaction of Cl⁻ and Cu nuclei surfaces might also be involved, which could reduce growth rates and generate a highly anisotropic growth for Cu nanocubes, similar to the ‘poisoning’ mechanisms for the anisotropic growth of other materials (Almeida and Alcacer, 1983).

In the absence of organic additives, the cathodic electrodeposition of Cu nanocubes can be described as:

\[
\text{Cu}^{2+} + 2\text{Cl}^- + e^- = \text{CuCl}_2^- \\
\text{CuCl}_2^- + e^- = \text{Cu} + \text{Cl}^- 
\]
The MWCNTs array also contributes to the formation of the Cu nanocubes because its 3D network provides large surface area and many electroactive sites for spatial diffusion, nucleation, and growth of Cu nanocubes. This hypothesis is supported by our experimental result that electrodeposition of Cu onto GC surface under the same conditions was hardly cubical because GC does not allow anisotropic growth in six facets.

3.2. Electrochemical characterization

In the Nyquist plot in the frequency range of 0.1 Hz to 100 kHz (Fig. 1F), the MWCNTs nearly show a straight line indicating Warburg impedance and the diffusion-limiting step in the electrochemical process (Ren and Pickup, 1997). In contrast, another similar straight line is seen for Cu-MWCNTs nanocomposite electrode suggesting the electrodeposition of Cu nanocubes did not hinder the electron transfer ability of MWCNTs. In the high frequency region, it can be easily inferred that the internal resistance is smaller after modification of Cu nanocubes than before modification, indicating the role of Cu nanocubes in the electron transfer process is that of an electron mediator and can therefore increase the heterogeneous electron transfer capability (Ye et al., 2005).

The voltammetric response of the MWCNTs was studied to estimate the electrochemical properties and effective surface area of the synthesized MWCNTs (Fig. S1). The potential peak difference $\Delta E_p$ is around 59 mV for MWCNTs electrode at sweep rates from 20 mV s$^{-1}$ to 500 mV s$^{-1}$ (Fig. S1 inset), which corresponds well to the theoretical value of 59 mV at 25°C for one-electron transfer pure-Nernstian behavior, revealing good conductivity and an...
ideal reversibility (Nugent et al., 2001) of redox reactions for the as-
synthesize MWCNTs. According to the Randles–Sevcik equation:

\[ I_p = 8.51 \times 10^{-3} n^{3/2} A D^{1/2} v^{1/2} C \]

The calculated effective area of MWCNTs is 0.064 cm². Notice-
ably in our experiments, the effective area of the MWCNTs array
barely changed after modification with Cu nanocubes.

3.3. Optimization of the sensor

Based on the dependence of the peak current and potential of
the catalytic oxidation of 1.0 mM Glc on NaOH concentrations at
the Cu–MWCNTs electrode by CV (Fig. S2), 0.1 M NaOH (pH = 13.0) was
selected as the optimal concentration because of its satisfactory
peak current and low oxidation potential.

The optimal detection potential was determined by dropwise
addition of 0.1 mM Glc in 0.1 M NaOH at 100 mV s⁻¹, with the
range of 0.1–0.6 V detection potentials applied (Fig. S3). Consid-
ering that the highest current response and the fact that high
potential may oxidize many unwanted species or intermediate interferents, +0.55 V was selected as the optimal detection potential.

3.4. Analytical performance of the sensor

Fig. 2A shows the typical voltammetric responses for the elec-
trodes. No oxidation peaks were observed in the absence of Glc
for both electrodes. When Glc was present, a negligibly small cur-
rent response was obtained for MWCNTs (Fig. 2A, c). This small response is probably due to the carbon nanotubes or the contribu-
tions from tiny amount of Co catalyst remaining after the synthesis
of MWCNTs (Ye et al., 2004). It has been frequently reported that
the electrochemical catalytic activities of MWCNTs were mainly
caused by the impurities of metal or metal oxide in the synthesiz-
ed MWCNTs (Batchelor-McAuley et al., 2008b). As for the Cu–MWCNTs
electrodes, their current responses increased rapidly and exhib-
ted obvious anodic oxidation peaks starting from 0.35 V to 0.65 V
in response to the addition of Glc (Fig. 2A, a). This suggests that
Cu is responsible for the Glc oxidation, with its catalytic activity
against Glc and large surface area of the nanocubes. The electro-
catalytic oxidation of glucose by metallic copper is via multiple steps including the formation of strong oxidizing Cu(III) species
(Wei et al., 2009). Therefore, the current response of Cu–MWCNTs
electrode is much higher than that of the MWCNTs electrode. This higher current response for Cu–MWCNTs electrode not only establishes its superiority over the unmodified MWCNTs electrode but also confirms the important role the Cu nanocubes plays in the electrocatalytic performance towards Glc oxidation due to the elec-
troactive sites afforded by the Cu nanostructure. In addition, no
obvious cathodic peaks were observed, i.e., the oxidation of Glc by
Cu is irreversible. As shown by linear sweep voltammetry (LSV)
at the Cu–MWCNTs electrode (Fig. S4), a gradual increase in the
current was measured with increased Glc concentration, pointing
to its potential for developing Cu–MWCNTs electrode as a generic non–enzymatic sensor.

Cyclic voltammograms of Cu–MWCNTs electrode in 0.1 M NaOH
with 1.0 mM Glc were recorded at different scan rates (Fig. 2B).
The anodic peak current (Iₚ) due to oxidation of Glc increased with increased scan rates but the anodic peak potential shifted
to positive values with increased scan rates. The Iₚ vs. scan rate,
ν (from 20 mV s⁻¹ to 500 mV s⁻¹) plot (Fig. 2B, inset) was highly
linear (R² = 0.993): Iₚ = 88.25 + 0.769ν. The result verifies that the
electrochemical kinetics is controlled by surface adsorption of Glc
molecules.

3.5. Amperometric response of the nanocomposite sensor
towards Glc

The amperometric responses of MWCNTs and Cu–MWCNTs
electrodes for a successive addition of 0.2 mM Glc at 50 s inter-
vals in 0.1 M NaOH at optimal potential of +0.55 V are shown in
Fig. 3A. As expected, the MWCNTs electrode shows extremely low
current response, and the Cu–MWCNTs electrode produced significa-
tively higher signals and generated steady-state current within one
second (Fig. 3A, inset). This extraordinarily fast response concurs
with fast diffusion of Glc molecules in the 3D network of MWC-
NTs arrays–Cu’s and fast electron transfers. The response is much
faster than those reported for Cu nanocluster/MWCNTs/GC (<5 s)
(Kang et al., 2007), SWCNTs/Cu/Nf (10 s) (Male et al., 2004), MWC-
NTs electrode (<10 s) (Ye et al., 2003). As shown in Fig. 3B, the
current response, i (µA) vs. Glc concentration C (mM) was linear
(R² = 0.996) up to C = 7.5 mM: i = 26.60 + 70.16C. Thus, the sensitivity
of Cu–MWCNTs electrode is 1096 µA mM⁻¹ cm⁻², and the detection
limit is 1.0 µM with an S/N of 3. The key performance characteris-
tics of different non–enzymatic Glc sensors from a copper source
are compared in Table S1. Among these, our Cu–MWCNTs sensor
boasts the best sensitivity and widest linear range. The wide lin-
ear range may be due to the high surface-to-volume ratio with
more electroactive sites and larger surface area for Glc molecules.
3.6. Stability and specificity of the sensor

The sensor shows a good stability, with a loss of only 12% in current response after 2 weeks (Fig. 3C). The reproducibility was examined for five identically-made electrodes with a relative standard deviation (RSD) of 4.3% while 10 measurements for the same electrodes were made upon the addition of 0.1 mM Glc in 0.1 M NaOH with RSD of 6.4%, demonstrating excellent reproducibility. Non-enzymatic sensors based on metals (Reitz et al., 2008) or alloys (Sun et al., 2001) could easily lose their electroactivity in the presence of Cl\(^{-}\), due to the formation of CuCl and/or CuCl complexes (Yuan et al., 2009). However, we did not observe any loss in electroactivity with our Cu-MWCNTs electrode while sensing Glc in 0.1 M NaOH solution with 0.1 M KCl; the current response remained almost unchanged, confirming excellent stability of our sensor against chloride poisoning.

A number of oxidizable interfering species could co-exist with Glc in many samples. Therefore, it is necessary to investigate if such interferents could generate current responses comparable to that corresponding to Glc. The normal physiological level of Glc in human blood is 3–8 mM compared to about 0.1 mM of interfering species (with Glc:interferents ratio of more than 30:1) (Zhang et al., 2008), while the Glc:interferents ratio is even higher in food samples. The measured effects of different interferents along with Glc in a carrier solution of 0.1 M NaOH at +0.55 V of operating potential are shown in Fig. 3C (inset). The current responses of all the interferents tested, except citric acid, were less than 5% (Table S2). The citric acid, a weak organic acid, which is usually a preservative in soft drinks and other foods, had a slightly higher influence of 7.4%, but the interference was still very minimal. These results indicate that Cu-MWCNTs electrode is highly specific to Glc even in the presence of several interfering species normally found in food and biological samples.

3.7. Testing Cu-MWCNTs performance

The performance of the Cu-MWCNTs electrode was tested by measuring Glc content in human serum blood samples obtained in a hospital for fasting blood sugar test from five patients (four non-diabetic and one diabetic) within 30 min of drawing the blood. 20 μL blood sample was added to a 20 mL 100 mM NaOH test solution and the optimal detection potential of +0.55 V was applied to record the current responses (Table S3). These results corresponded well with hospital-conducted blood test results on a commercial instrument. The blood Glc concentrations of non-diabetic patients were between 4.44 mM and 5.16 mM, which are in the normal range of 3–8 mM; the diabetic patient had a blood Glc level of 12.73 mM. For all the tests, 0.1 mM standard Glc was added to calculate the recovery rate and our sensor exhibited a good recovery (>90%). These results validate the potential of our Cu-MWCNTs electrode to be used for routine blood Glc sensing.

4. Conclusions

A novel amperometric Glc sensor has been fabricated based on a simple one-step potentiostatic electrodeposition of Cu's onto the vertically well-aligned MWCNTs. The Cu-MWCNTs electrode exhibited excellent sensitivity (1096 μA mM\(^{-1}\) cm\(^{-2}\)), high stability, low detection limit (1 μM), and fast response (<1 s). The linear range of our sensor is 7.5 mM of Glc, which is greater than that of most of non-enzymatic Glc sensors from a copper source. The sensor exhibited high specificity to blood Glc in the presence of many common interfering agents and performed very well in measuring blood Glc levels of diabetic and non-diabetic patients. The relatively facile and low-cost fabrication of Cu-MWCNTs electrode combined with its many desirable performance characteristics make it an excellent choice for routine Glc sensing.
Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2010.06.014.

References