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Short communication

3D graphene foams decorated by CuO nanoflowers for ultrasensitive ascorbic acid detection

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ABSTRACT

When the in vitro research works of biosensing begin to mimic in vivo conditions, some certain three-dimensional (3D) structures of biosensors are needed to accommodate biomolecules, bacteria or even cells to resemble the in vivo 3D environment. To meet this end, a novel method of synthesizing CuO nanoflowers on the 3D graphene foam (GF) was first demonstrated. The 3DGF/CuO nanoflowers composite was used as a monolithic free-standing 3D biosensor for electrochemical detection of ascorbic acid (AA). The 3D conductive structure of the GF is favorable for current collection, mass transport and loading bioactive chemicals. And CuO nanoflowers further increase the active surface area and catalyze the redox of AA. Thus, all these features endows 3DGF/CuO composite with outstanding biosensing properties such as an ultrahigh sensitivity of 2.06 mA mM^{-1} cm^{-2} to AA at 3 s response time.

1. Introduction

Cells, bacteria and many other microbes are inherently sensitive to local macroscale, microscale and nanoscale three-dimensional (3D) environment and the adherence to a 3D structure is often needed (da Rocha-Azevedo and Grinnell, 2013; Muller et al., 2013). With the advancements of in vitro research of biomolecule detections, drug delivery, tissue culture and microbial fuel cells, researchers begin to focus on mimicking the in vivo condition to make sure that their results are still reliable when applied to the in vivo process of bio-metabolism (Nirantar et al., 2013; Rawson et al., 2013; Zhao et al., 2013). It would be more attractive to associate the level of analytes in the solution with the quantity and health condition of cells and bacteria all by one integrated biosensor. Therefore, a 3D structure is essential for designing the new generation biosensors. But the traditional biosensors are mostly 1D or 2D devices that the electrocatalytic materials are often attached to an inactive electrode to detect the analytes and cannot be easily shaped into three dimensions (Wang, 2008) to support bioactive molecules. Meanwhile, the traditional scaffolds can provide a bio-friendly 3D morphology but cannot provide sufficient feedbacks concerning the level of analytes due to the poor conductivity of scaffolds (Grafahrend et al., 2011). So a bioactive 3D biosensor is expected to meet both the needs.

Graphene, a two-dimensional monolayer of sp2 carbon atoms, has drawn numerous interest in this decade owning to its large specific surface area, unusual mechanical strength, outstanding electrical properties and good bio-compatibility (Reina et al., 2008; Tao et al., 2013; Zhang et al., 2013b). In particular, many research works have focused on graphene-based materials for high-performance biosensors (Pumera, 2011; Sun et al., 2011b; Wu et al., 2013; Yang et al., 2010). However, the conventional grown graphene is always a two-dimensional sheet and its morphology cannot be easily changed which limits the active surface area and further applications (Huang et al., 2011). Therefore, three-dimensional graphene foams (3DGF), a seamlessly connected porous carbon network can desirably tackle these problems to be an ideal conductive scaffold to replace the traditional supporting electrodes.

Ascorbic acid (AA), also known as vitamin C is a common water soluble pharmaceutical compound usually used to prevent or treat some diseases in public. AA is vital to many human metabolism processes such as enhancing iron uptake in human intestinal cells, involving in immune cell functions and immune responses and inducing differentiation of cells (Kim et al., 2012; Temu et al., 2010). Besides, bacteria also have close relationship with AA in food production and even the AA production itself (Dave and Shah, 1997). So a simple and rapid detection method of AA under complex condition is needed for biomedical chemistry, diagnostics and pathological research.

CuO, a transition metal oxide is under tremendous attention as biosensing materials due to their low cost, ease of synthesis, chemical stability and outstanding redox behavior at various

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potential ranges under diverse reaction conditions. So CuO has been widely used as catalysis, semiconductors, gas sensors and Li-ion rechargeable batteries (Dubal et al., 2013; Steinhauer et al., 2013; Sun et al., 2013). Some previous studies have shown that CuO has notable electrochemical properties of catalyzing biomolecules, therefore predict direct electrochemical detection of AA without the need of any other mediators (Hsu et al., 2012; Jiang and Zhang, 2010; Jindal et al., 2012). Moreover, the variable nanostructures of CuO could further enlarge the specific surface area and the quantity of active sites to obtain impressive sensitivity.

In this study, we demonstrate a 3DGF/CuO nanoflowers composite as a novel free-standing and monolithic biosensor with large specific surface area, high conductive pathways, well-organized 3D porous structure and ultrahigh sensitivity for AA. The 3D morphology and high sensitivity make it a promising biosensor for in vitro detection mimicking in vivo condition in the future.

2. Experimental

2.1. Fabrication of 3D graphene foam

As described in previous study (Chen et al., 2011), the 3DGF was synthesized by CVD on 1.5 mm × 1 cm × 1 cm nickel foam under atmosphere pressure. The samples were first heated to 1000 °C with the flow of H_2 and Ar (H_2/Ar=150:300 sccm). Then 15 sccm CH_4 was introduced into the quartz tube to synthesize the graphene in 15 min. After the growth, samples were rapidly cooled down to room temperature in 10 min with the protection of H_2 and Ar. At last, the Ni/GF were immersed into 3 M HCl solution at 80 °C to etch away the nickel foam to get the free-standing 3DGF.

2.2. Synthesis of 3D graphene and CuO nanoflowers composite

First, the Cu nanoparticles were electrodeposited on the 3D graphene foam by maintaining potential −0.40 V for 30 s in 0.1 M H_2SO_4, 0.01 M $CuSO_4$ solution. The samples served as the working electrode in a three-electrode system. Then the sample was immersed into a solution of 0.05 M $K_2S_2O_8$, 1.5 M NaOH at 80 °C for 5 min to get the final 3DGF/CuO nanoflowers composite.

2.3. Synthesis of CuO electrode

Similar to the synthesis of 3DGF/CuO nanoflowers, Cu nanoparticles were electrodeposited on the glass carbon electrode (GCE, diameter 3 mm) then oxidized into CuO nanoflowers.

2.4. Characterization

The morphology of samples was characterized with HITACHI S-4800 electron microscopy (SEM). Energy-dispersive X-ray spectroscopy (EDX) was tested by EDAX Gernesis. Raman spectra were recorded at ambient temperature on a Labor Raman HR-800 system with 514.532 nm wavelength laser. The X-ray diffraction (XRD) was carried out on a PANalytical B.V. Empyrean 200895 (Netherland) using Cu Kα radiation.

2.5. Electrochemical measurements

The electrochemical impedance spectroscopy (EIS) was performed with CHI 760e (U.S.). The other electrochemical experiments were carried out with a WPG100e electrochemical workstation (Korea). A three-electrode system was employed in all electrochemical experiments. Rinsed by ethanol and water twice, the 3DGF or 3DGF/CuO were griped by a Pt clip which is connected to the electrochemical workstation. The 3DGF or 3DGF/CuO composite served as the working electrode, while a platinum electrode and a SCE electrode were used as the counter and reference electrodes, respectively.

3. Results and discussion

3.1. Characterization of 3DGF/CuO

As shown in Fig. 1a, the as prepared 3DGF/CuO composite is much darker than the 3DGF in color, indicating that the CuO nanoflowers have been evenly anchored on the surface of 3DGF. The structure and morphology are then studied by scanning electron microscopy (SEM, Fig. 1b–f). The bare 3DGF is a smooth and macro-porous graphene skeleton with wrinkles distributed on the surface (Fig. 1b). The hollow graphene bridge is about 50 μm wide with no cracks or breaks and the diameter of pores ranges from 100 μm to 300 μm. As to the 3DGF/CuO composite, Fig. 1c–f show that the 3DGF is uniformly covered by CuO nanoflowers which is about 400 nm in diameter with thin nanoflakes (about 10 nm thick, 50 nm wide and 200 nm long) as petals (Fig. 1f). The 3DGF provides an ideal morphology for anchoring bioactive molecules and rapid pathway for mass transfer, while CuO nanoflowers offer a large accessible surface area and numerous chemical active sites.

The XRD pattern is shown in Fig. 1g. All peaks can be assigned to either 3DGF or CuO, which shows no impurity phase exists and good crystallinity of CuO. The Raman spectra of graphene foam (Fig. 1h) show two characteristic peaks at 1581 cm^{-1} and 2725 cm^{-1} which can be attributed to G and 2D peaks. No peaks at 1350 cm^{-1} indicate good quality and the lack of defects (Malard et al., 2009). The elemental purity of the 3DGF/CuO composite was also confirmed by EDX (Fig. 1h inset), showing only C, O, and Cu in the sample.

3.2. Electrochemical properties

First, the electrochemical active surface areas (ECSA) of CuO/GCE, 3DGF and 3DGF/CuO were calculated as 0.0861 cm^2, 3.99 cm^2 and 8.98 cm^2, according to the Randles–Sevcik equation (Lu et al., 2008):

$$I_p = 268600\, n^{2/3} A D^{1/2} C v^{1/2}$$

where I_p is current maximum in amps, n number of electrons transferred in the redox event, A the electrochemical active area in cm^2, D diffusion coefficient in cm^2/s (6.70×10^{-6} cm^2/s), C concentration in mol/L (0.005 M $K_3Fe(CN)_6$) and v scan rate in V/s. The relative standard deviation (R.S.D.) of ECSA among three 3DGF/CuO sensors is 5.7%. The merit that 3DGF/CuO has the largest ECSA promises its good biosensing property.

The electrochemical properties of 3DGF/CuO composite were then examined by EIS in 0.1 M phosphate buffer solution (PBS, pH 7.4) in the frequency range from 0.1 Hz to 100 kHz. As shown in the Nyquist plots (Fig. 2a), the resistance of 3DGF/CuO (20 Ω) is much smaller than that of bare 3DGF (28 Ω) and CuO on GCE (157 Ω) in the high frequency domain, indicating that the 3DGF/CuO has higher electrochemical activity and faster electron transfers on the surface than the bare 3DGF and CuO (Wang et al., 2010).

Fig. 2b shows the different cyclic voltammetry (CV) response of CuO on GCE, the bare 3DGF and 3DGF/CuO in 40 μM AA and 0.1 M phosphate buffer solution at the scan rate of 50 mV/s from −0.8 V to 0.4 V. The dramatically enhanced CV curve of 3DGF/CuO indicates the high electrocatalytic activity of CuO nanoflowers comparing with the bare 3DGF electrode and fast charge collecting