Fabrication of multianalyte CeO2 nanograin electrolyte–insulator–semiconductor biosensors by using CF4 plasma treatment

Chyuan Haur Kao
Chang Gang University

Hsiang Chen
National Chi Nan University

Fang Yao Stephen Hou
Marquette University, fangyaostephen.hou@marquette.edu

Shan Wei Chang
Chang Gang University

Che Wei Chang
Chang Gang University

See next page for additional authors

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Chyuan Haur Kao\textsuperscript{a,d}, Hsiang Chen\textsuperscript{b,x}, Fang Yao Stephen Hou\textsuperscript{c}, Shan Wei Chang\textsuperscript{a,d}, Che Wei Chang\textsuperscript{a,d}, Chao Sung Lai\textsuperscript{a,d}, Chin Pang Chen\textsuperscript{b}, Yun Yang He\textsuperscript{b}, Shang-Ren Lin\textsuperscript{b}, Kun Min Hsieh\textsuperscript{b}, Min Han Lin\textsuperscript{b}

\textsuperscript{a}Department of Electronic Engineering, Chang Gung University, Taiwan, ROC
\textsuperscript{b}Department of Applied Materials and Optoelectronic Engineering, National Chi Nan University, Taiwan, ROC
\textsuperscript{c}Clinical Laboratory, Marquette University, USA
\textsuperscript{d}Center for Biomedical Engineering, Chang Gung University, Taiwan, ROC

1. Introduction

Over the past 10 years nanostructure-based biosensors have been intensively studied because of their distinct sensing capability \cite{1,2}. It is commonly acknowledged that the biosensing properties of these sensors are not only relevant to their dimensions but also to their shapes \cite{3-5}. Therefore, managing the morphologies of these sensors are not only relevant to their dimensions but also to their shapes \cite{3-5}. Shape-controlled fabrication processes have been demonstrated, and nanostructures with various shapes such as cubes, wires, and rods have been designed using various synthesis techniques \cite{8-10}. Because the surface contact area of a membrane can be enlarged and the number of the surface sites can be increased, biosensors with desirable morphologies can achieve high sensitivity and linearity \cite{11,12}. Shape-controlled fabrication processes have been demonstrated, and nanostructures with various shapes such as cubes, wires, and rods have been designed using various synthesis techniques \cite{8-10}. Recently, EIS structures have been intensively investigated because of their simple structure and fast detection \cite{13,14}. To enhance multianalyte sensing performance, various materials, alternative processes, and treatments such as annealing have been incorporated \cite{15,16}. However, multianalyte biosensing EIS membranes with nanostructures on the membrane have not yet been clearly reported. In this research, we introduced CF\textsubscript{4} plasma treatment to shape nanostructures and, thus, increase the surface area and the number of surface sites of the membrane \cite{17}. Nanostructures formed using plasma treatment were observed in atomic force microscope (AFM) and scanning electron microscope (SEM) images \cite{18}. Secondary ion mass spectroscopy (SIMS) results indicated that fluorine atoms on the membrane surface might be related to the formation of nanostructures. Consistent with the AFM and SEM images, X-ray diffraction (XRD) patterns and X-ray photoelectron spectroscopy (XPS) measurements confirmed that crystallization and grainization of CeO\textsubscript{2} might cause the formation of nanostructures \cite{19}. The CeO\textsubscript{2} multianalyte biosensor demonstrated multiple sensing capability with solutions containing H\textsuperscript{+}, Na\textsuperscript{+}, K\textsuperscript{+}, glucose \cite{20}, and urea \cite{21}. Furthermore, the results indicated that the formation of nanograins on the CeO\textsubscript{2} membrane further enhanced the multianalyte sensing capability of various electrolytes.

The impact of the development of nanograins on the understanding of the detecting film can be illustrated by considering the site-binding design in an explanation of the ionic consumption procedures at the electrolyte/oxide interface \cite{13}. For pH detecting ability, the reaction voltage relies on the outer lining area prospective (\psi), which is an operation of the membrane content and the pH of the electrolyte \cite{13,22}. The value of (\psi) can be calculated using Eq. (2).
The influence of the formation of nanograins on the detection of the sensing film can be understood by considering the site-binding model. To illustrate the ionic absorption procedures at the electrolyte/oxide interface involved in pH sensing, the detecting voltage on the surface potential ($\psi$) can be expressed as a function of the membrane material and the pH value of the electrolyte. The value of ($\psi$) can be calculated using Eq. (1).

$$\psi = \frac{2 \sqrt{3} kT}{q} \frac{\beta}{\beta + 1} (\text{pH}_{\text{pzc}} - \text{pH})$$

where $k$ is Boltzmann’s constant, $T$ is the temperature, $q$ is the elementary charge, $\text{pH}_{\text{pzc}}$ is the pH value with zero charge, and $\beta$ is a factor that signifies the chemical sensitivity of the gate oxide. Furthermore, the $\beta$ value is proportional to the chemical sensitivity of the gate dielectric and is determined according to the density of surface hydroxyl groups, as described in Eq. (2).

$$\beta = \frac{2q^2N_s\sqrt{K_aK_b}}{KT_{\text{DL}}}$$

where $N_s$ is the count of surface site per unit area and $C_{\text{DL}}$ is the double layer capacitance based on the Gouy–Chapman–Stern model [23]. According to Eqs. (1) and (2), $\beta$ can be expected to result in a higher sensitivity and linearity in accordance with the value revealed in the report [24]. Nanograins formed through plasma treatment can noticeably increase the surface area and, hence, the surface sites; therefore, the sensing performance can be enhanced by incorporating plasma treatment.

2. Experimental details

EIS biosensors containing CeO$_2$ membranes were designed on 4-inch n-type (100) Si wafers with a resistivity of 5–10 $\Omega$-cm. After RCA cleaning procedure, the wafers were dropped into 1% hydrofluoric acid to eliminate oxide from the surface. First, a 50-nm CeO$_2$ membrane was placed on the Si substrate through radio frequency (rf) sputtering from a cerium target in a diluted O$_2$ ambient (Ar/O$_2$ = 20 sccm/5 sccm). The rf power and ambient pressure were 100 W and 20 mTorr, respectively. After deposition, CeO$_2$ membrane layers were subjected to a post-CF$_4$ treatment in a plasma-enhanced chemical vapor deposition system with an rf power of 30 W and a processing pressure of 500 mTorr for 15, 30, and 60 s, respectively. Subsequently, the back-side contact of the Si wafer was grown using a 300-nm Al film. The size of the sensing membrane was defined through photolithographic processing under a photosensitive epoxy (SU8-2005, Micro-Chem). EIS structures were then placed on the copper lines of a printed circuit board by using a silver gel. An epoxy package was included to separate the EIS structure and the copper line. The device structure is shown in the supplement data.
To examine the material properties of the sensing membrane, the CeO$_2$ membrane layer was analyzed using FESEM and AFM to investigate the nanograins. In addition, XRD and XPS were used to examine the crystalline structure and chemical bonds of the membrane layer. After the biosensor was fabricated and packed, the capacitance–voltage (C–V) curve was measured. To evaluate the sensing performance from the C–V curve, the responsive voltages for all devices were calculated using $0.4 \times C_{\text{max}}$ ($C_{\text{max}}$: maximum capacitance value in the C–V curve). We used $0.4 \times C_{\text{max}}$ as a standard line in the C–V curve and calculated the substrate-bias voltage changes after the device was submerged in solutions with various pH values. FESEM images were measured using a SM-7500F JEOL cold-field emission SEM. AFM images were taken using a Veeco D5000 AFM in tapping mode employing an applied nanosilicon tip with a 50-N/m spring constant. XRD was performed using XRD Bede D1. The X-ray source was Cu Kα ($\lambda = 1.542$ Å), and the XRD configuration was the Bragg–Brentano theta-2 theta. The diffraction angle 2$\theta$ ranged from 20° to 80° with a grazing incidence angle $\theta$ of 0.5°. The XPS spectra were performed using a VG ESCA Scientific Theta Probe (2002). The X-ray spot size, the take-off angle, and the pass energy were 15 µm, 53°, and 50 eV, respectively. The X-ray source for XPS was Al Kα (1486.6 eV). The C–V curves were measured using a precision LCR meter (HP-4284, Hewlett-Packard, USA).

3. Results and discussion

3.1. Material characterizations

To examine nanograins on the membrane surface, FESEM was used to reveal the surface morphology of the CeO$_2$ membrane layer before deposition of the metal contact. Before the CF$_4$ plasma treatment, the membrane surface was flat and no protruded nanostructures were observed, as shown in Fig. 1(a). After the film was treated with CF$_4$ plasma for 15 s, the nanograins on the surface formed, as shown in Fig. 1(b). The average size of a nanograin was 15 nm, and most of the grains exhibited a similar size, indicating that the CF$_4$ plasma could form uniform nanograins on the membrane surface. Consistent with the FESEM images, AFM analysis
revealed that uniform nanograins were generated on the membrane surface. After CF₄ plasma treatment, the membrane surface became rougher and nanograins were observed on the membrane surface, as shown in the AFM images in Fig. 1(c) and (d). The formation of the nanograin structures increased the surface area of the CeO₂ membrane. The number of surface sites, which is related to sensing capability, also increased.

To gain insight into the influence of CF₄ plasma treatment, SIMS measurement was performed. SIMS results, as shown in Fig. 2, revealed that fluorine atoms accumulated on the surface and piled up near the CeO₂/Si interface. The accumulation of fluorine atoms near the surface might result in the formation of Ce–F bonds and crystallization of the CeO₂ film near the surface as shown in the XRD and XPS analysis in the supplement information. Therefore, the incorporated fluorine atoms might have caused the formation of the CeO₂ nanograins owing to enhancement of binding. Furthermore, the fluorine atoms piled up near the CeO₂/Si might repair the dangling bonds, traps, and the defects near the interface and, thus, enhance the sensing performance of the CeO₂ membrane.

To investigate the sensing performance of the CeO₂ nanograin EIS biosensor, the pH sensitivity, pH linearity, hysteresis voltage, and drift rate were evaluated.

3.2. pH sensitivity and linearity

Fig. 3(a–d) show the C–V curves of the CeO₂ membrane and the sensing membrane treated with CF₄ plasma for 15, 30, and 60 s in a buffer solution with various pH values. The threshold voltage shift of the CeO₂ membranes revealed the sensitivity of the device and was as high as 55.88 mV/pH. The sensitivities of the as-deposited membrane and the CeO₂ membranes that underwent plasma treatment for 15, 30, and 60 s were 43.20, 53.38, 43.78, and 37.23 mV/pH, respectively, as shown in the supplement data. As observed in the material analysis, the CeO₂ treated in plasma for 15 s exhibited the highest sensitivity. Furthermore, the pH sensing membrane that underwent CF₄ plasma treatment for 15 s exhibited excellent linearity of 99.83. This was attributed to the formation of nanograins on the surface CeO₂ membrane after plasma processing. The formation of the nanograins enlarged the
membrane surface contact area and increased the surface sites. Therefore, the sensitivity and linearity were noticeably enhanced. However, as the plasma treatment time increased to 30 and 60 s, the sensing performance deteriorated; the material analysis revealed that the long plasma treatment damaged the surface. The hysteresis voltage, and drift rate are presented in the supplement data.

3.3. Multianalyte sensing properties

In addition to pH sensing, the concentration of biologically relevant ions is a crucial indicator associated with patient heath conditions. Therefore, various methods for detecting potassium ion (K⁺) and sodium ion (Na⁺) concentrations have been employed in biomedical research [25]. In addition, K⁺ and Na⁺ ion concentrations are crucial indicators of food and wine quality and water pollution [26].

The K⁺ ion and Na⁺ ion sensing properties of the CeO₂ membranes were analyzed. We measured the sensitivity of the as-deposited CeO₂ sensing membrane and the membrane treated with CF₄ plasma for 15 s. First, a 5 mM Tris/HCl buffer solution, of which the pH value was kept at 8.7, was prepared [27]. A micropipette was used to control the concentrations of Na⁺ and K⁺ ions in the range between 10⁻⁵ to 10⁻¹ M while 1 M NaCl/Tris–HCl and 1 M KCl/Tris–HCl were injected into the buffer electrolyte. Subsequently, the pNa sensitivity of the as-deposited CeO₂ membrane and the CeO₂ membrane treated with plasma for 15 s was calculated, as shown in Fig. 4(a) and (b). Moreover, the pK sensitivity of the as-deposited CeO₂ membrane and the CeO₂ membrane treated with plasma for 15 s was calculated, as shown in Fig. 4(c) and (d). The pNa and pK sensitivity values of the as-deposited CeO₂ sensing membrane were 11.68 mV/pNa and 9.78 mV/pK, respectively. After the membrane was treated with CF₄ plasma for 15 s, the pNa and pK sensitivity of the sensing membrane were increased to 13.83 mV/pNa and 13.03 mV/pK, respectively. The EIS structure containing nanograins was more sensitive to Na⁺ and K⁺ than that without nanostructures.

A CeO₂ nanograin sensing membrane on an EIS structure was used to detect urine concentrations in solutions. Monitoring urea concentrations by using biosensors is of great interest in biomedical applications. The concentration of urea in blood is a crucial indicator of patient constitution and used in disease evaluation.
Because enzymatic reactions usually produce ionic species, selective detection of various chemicals involves using ion-selective membranes with appropriate enzymes. To stabilize biosensor properties and lengthen their lifetimes, various methods of enzyme immobilization, such as physical adsorption [28], entrapment [30], covalent bonding [29], and cross-linking have been proposed. In this study, we established a process for immobilizing urease by using covalent bonding methods [31]. The developed CeO$_2$ nanograin EIS biosensor facilitated investigating the pUrea sensing performance.

The following is the equation of the urea hydrolysis reaction catalyzed by urease:

$$\text{NH}_2\text{CONH}_2 + 3\text{H}_2\text{O} \rightarrow 2\text{NH}_4^+ + \text{OH}^- + \text{HCO}_3^-$$

The generated hydrogen ions and ammonium ions can be used for urea detection. The variation of the pH value caused by an enzyme-catalyzed reaction can lead to changes in the analytical output signal of a sensor.

To study the urea sensing properties of the CeO$_2$ nanograin sensing membrane, the urea sensitivity and linearity were measured in solutions in which the urea concentration was controlled in a range between 5 and 40 mM. As shown in Fig. 5(a) and (b), the as-deposited CeO$_2$ membranes exhibited a urea sensitivity of 1.33 mV/mM and linearity of 89.19%; the CeO$_2$ membranes with nanostructures after CF$_4$ plasma treatment exhibited a higher sensitivity of 2.30 mV/mM and a higher linearity of 94.58%. The results indicated that incorporating nanostructures onto the membrane surface enhanced the urea sensing performance as well as the capability to sense H$^+$, Na$^+$, and K$^+$. We used an enzymatic EIS-based membrane similar to that used in urea detection for glucose detection. Glucose oxidase was used as the biocomponent, and the biosensor measured the glucose concentration by measuring the changes in pH values caused by the generation of hydrogen ions with the dissociation of glucose acid. The chemical formula for the hydrolysis of glucose by glucose oxidase is as follows:

$$\beta-\text{D-Glucose} + \text{O}_2 + \text{H}_2\text{O} \rightarrow \text{D-glucose-}\delta-\text{lactone} + \text{H}_2\text{O}_2$$

Similarly, the procedure of glucose immobilization was achieved by employing covalent bonding methods. To examine the glucose sensing properties, the CeO$_2$ sensing membrane on the EIS structure was used to measure the glucose concentrations in solutions in which the glucose concentration was controlled in a range between 2 and 7 mM. As shown in Fig. 5(c) and (d), the as-deposited CeO$_2$ membranes exhibited a low glucose sensitivity of 3.46 mV/mM and linearity of 93.76%. After the membrane was incorporated with nanostructures through plasma treatment, the sensitivity increased to 5.83 mV/mM and the linearity increased to 94.58%. The results revealed that nanostructures on the membrane enhanced the multianalyte sensing performance because of the increase of the contact area and the surface sites and suppression of defects. After the nanostructures were incorporated through CF$_4$ plasma treatment, the capability to sense multiple analytes, namely H$^+$, Na$^+$, K$^+$, urea, and glucose, was improved.

4. Conclusion

In this study, multianalyte CeO$_2$ nanograin EIS biosensors were fabricated. Nanograin structures formed on top of the CeO$_2$ membrane surface, as shown in FESEM and AFM images. The formation of nanostructures through CF$_4$ plasma treatment for an appropriate time of 15 s was associated with enhanced crystalline structures and stronger chemical binding, as indicated by XRD and XPS measurements. Furthermore, adding fluorine atoms during the plasma treatment process might have been related to the formation of the nanostructures, as indicated by SIMS measurements. Therefore, incorporating nanostructures can enhance the performance of biosensors. A higher sensitivity, higher linearity, lower hysteresis voltage, and lower drift ratio of pH sensing was achieved. Moreover, the capability to sense multiple analytes, namely H$^+$, Na$^+$, K$^+$, urea, and glucose, was achieved by incorporating nanostructures onto the membrane surface, thereby increasing the membrane surface contact area and the effective surface sites. Multianalyte CeO$_2$ nanograin EIS biosensors exhibit potential for use in future biomedical sensing device applications.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.sbsr.2015.07.001.

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