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# Nanocrystalline 3C-SiC Electrode for Biosensing Applications

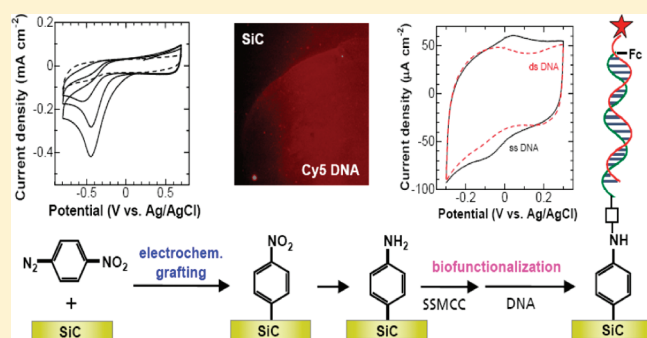
Nianjun Yang,<sup>\*,†</sup> Hao Zhuang,<sup>‡</sup> René Hoffmann,<sup>†</sup> Waldemar Smirnov,<sup>†</sup> Jakob Hees,<sup>†</sup> Xin Jiang,<sup>‡</sup> and Christoph E. Nebel<sup>†</sup>

<sup>†</sup>Fraunhofer Institute for Applied Solid State Physics (IAF), Tullastrasse 72, Freiburg 79108, Germany

<sup>‡</sup>Institute of Materials Engineering, University of Siegen, Paul-Bonatz-Strasse 9-11, Siegen 57076, Germany

**S** Supporting Information

**ABSTRACT:** Silicon carbide has been proved as a candidate for power and high-frequency devices. In this paper, we show the application of nanocrystalline 3C-SiC as an electrochemical electrode and its electrochemical functionalization for biosensing applications. SiC electrodes show a wider potential window and lower background current than glassy carbon electrodes. The surface can be electrochemically functionalized with diazonium salts, as confirmed by electrochemical techniques and X-ray photoelectron spectroscopy. The nitrophenyl film is used as linker layer to bond DNA molecule to SiC. These results show that 3C-SiC can be an interesting transducer material for applications in electro- and bioelectrochemical applications.



Silicon carbide (SiC) belongs to the class of wide bandgap semiconductors showing optical gaps between 2.4 and 3.2 eV, depending on the polytype (4H-, 6H-, 3C-) structure of SiC. It can be grown on a large area (>3 in.) either in single- or nanocrystalline form. It can be n- or p-type doped from insulating to semiconducting or metallic. It has high radiation and chemical hardness, high thermal conductivity (3.6–4.9 W cm<sup>-1</sup> K<sup>-1</sup>), high Young's modulus of typically 450 GPa compared to 130 GPa for Si, and a high breakdown electric field of typically larger than 2 MV cm<sup>-1</sup>. The outstanding electronic and mechanical properties of SiC are currently utilized in a wide range of applications such as high frequency and power devices.<sup>1,2</sup> Moreover, SiC is multifunctional, nontoxic, biocompatible, chemically stable, and transparent to visible light. It is, however, only recently recognized as candidate for biosensor platforms.<sup>3–6</sup> SiC based implantable sensors, porous membranes for protein separation, microelectrode arrays, and microfluidic systems have meanwhile been demonstrated.<sup>4–6</sup>

For high performance of bioelectronic devices and integrated systems, it is required to combine sophisticated electronic circuits with linker and biorecognition layers. Although the functionalization of a H-terminated SiC surface is expected to be possible and the different polytypes of SiC will match quite well to organic systems in terms of band-gaps and band-alignments,<sup>4</sup> only recently a theoretical discussion in combination with experimental activities of surface modification of SiC has been started.<sup>3,7–13</sup> *Ab initio* molecular simulations predicted the possibility to functionalize C- and Si-terminated SiC surfaces.<sup>7</sup> Direct adsorption of biotin molecules on bare and hydroxylated Si-terminated SiC (001) surfaces was theoretically studied.<sup>8</sup> Theoretical and experimental work on pyrrole

functionalization of Si- and C-terminated 6H-SiC surface was published.<sup>9</sup> New techniques were applied to modify the SiC surface, such as self-assembling of organosilanes on the 4H- and 6H-SiC surface,<sup>3,10</sup> and thermal<sup>11</sup> and photochemical<sup>12</sup> attachment of the 6H- and 3C-SiC surface with terminal alkenes. Functionalization of SiC nanoparticles has also been shown using azo radical initiators<sup>13</sup> as well as using plasma treatment.<sup>14</sup>

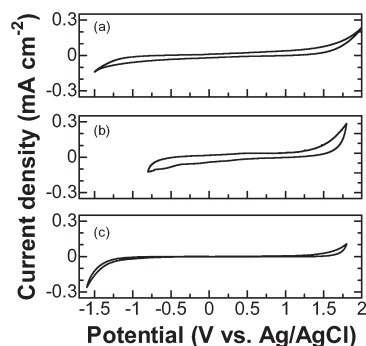
It has been proposed more than 70 years ago that an electrode made of single crystalline silica carbide behaves as a noble electrode, although it will be troublesome to get the right contact due to its high resistance.<sup>15</sup> Nowadays, nanocrystalline cubic (3C-) SiC exhibits a much higher electron mobility than single crystalline SiC<sup>16</sup> because its average oxygen concentration is enhanced and the oxygen is accumulated at crystal defects, resulting in a much better conductivity.<sup>17</sup> However, up until now, no investigation has been reported in the literature on the applications of nanocrystalline 3C-SiC as an electrode material or on the electrochemical surface modification of 3C-SiC.

The aim of this work is to explore electrochemical schemas of surface modifications of nanocrystalline 3C-SiC and to characterize further applications in bioelectronics like DNA sensors. We will introduce the electrochemical properties of nanocrystalline 3C-SiC and then show a novel electrochemical way to functionalize SiC with phenyl-linker molecules. To realize DNA sensing, we used well-established recipes to covalently bond DNA to the linker molecules and characterized the bonding properties.

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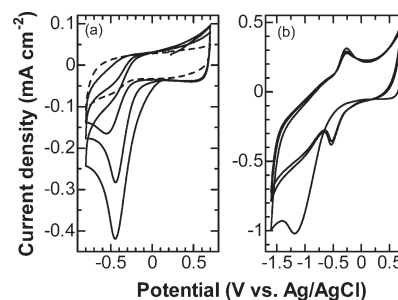


**Figure 1.** Cyclic voltammograms on a nanocrystalline 3C-SiC electrode (a), glassy carbon electrode (b), and boron-doped diamond electrode (c) in 0.1 M  $\text{H}_2\text{SO}_4$  at a scan rate of  $100 \text{ mV s}^{-1}$ . The boron concentration of diamond electrode is  $5 \times 10^{20} \text{ cm}^{-3}$ .

The nanocrystalline 3C-SiC film we grew was relatively rough with a room-mean-square roughness value of 30 nm, as measured by a contact-mode atomic force microscopy. The grain size of the film was 5–20 nm and the ratio of Si to C is 1:1, as detected by Rutherford backscattering spectrometry (RBS) and electron probe microanalysis (EPMA) measurement.<sup>18</sup> It was conductive and the resistivity was about 3.5–6.2 k $\Omega$  cm. The samples were cleaned to remove any inorganic and organic contaminations.<sup>19</sup>

To test the potential of nanocrystalline 3C-SiC as a candidate for electrochemistry, its electrochemical potential window, and the background current were characterized. Figure 1 shows voltammograms of a SiC electrode for water electrolysis, of a glassy carbon electrode, and of a boron-doped diamond electrode. We define a current density of  $0.1 \text{ mA cm}^{-2}$  as a threshold to deduce the potential windows for the three transducer materials. The electrochemical potential window of SiC is about 3.0 V, which is slightly narrower than that of boron-doped diamond (3.2 V) but much wider than that of glassy carbon (2.2 V). The background current of SiC is about 3 to 5 times smaller than glassy carbon but 20–50 times larger than boron-doped diamond. At the SiC electrode, voltammetric responses of redox couples like  $\text{Fe}(\text{CN})_6^{3-/4-}$  show redox waves with comparable peak current densities of  $0.35 \pm 10 \text{ mA cm}^{-2}$ , the formal potential  $E_{1/2} = 160 \pm 10 \text{ mV}$ , and the peak differences of anodic waves from cathodic one,  $\Delta E$ , of  $140 \pm 20 \text{ mV}$  measured at a scan rate of  $100 \text{ mV s}^{-1}$ . Subsequently, nanocrystalline 3C-SiC layers are promising as electrochemical electrodes. However, to compare the properties of nanocrystalline SiC electrode with previously reported robust electrode such as boron-doped diamond, the electrochemical response and reaction kinetics of other redox species such as  $\text{Fe}^{2+}/\text{Fe}^{3+}$ ,  $\text{Ru}(\text{NH}_3)_6^{2+/3+}$ , ferrocene-methanol ( $\text{FcOH}$ ), dopamine, etc. on this electrode need to be investigated.

Electrochemical reduction of aryl-diazonium salts at carbon based electrodes has been proven as a simple and versatile method to functionalize electrodes. The mechanism of this covalent attachment of nitrophenyl molecules proceeds via reductive formation of aryl radicals which form covalent bonds to the carbon dominated surface of SiC, resulting in stable C–C bonds.<sup>20</sup> Figure 2a shows voltammograms for the reduction of diazonium salts on a SiC electrode. An irreversible reduction peak centered around  $-440 \pm 60 \text{ mV}$  vs Ag/AgCl is detected during the first cycle. The cathodic peak potential shifts toward more negative potentials in subsequent cycles. The peak disappears

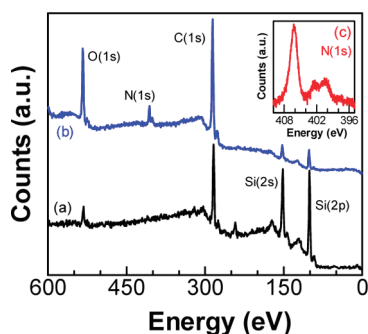


**Figure 2.** (a) Electrochemical grafting of SiC with 4-nitrobenzene diazonium tetrafluoroborate using cyclic voltammetry. The concentration of diazonium salts was 1.0 mM. The electrolyte was 0.1 M tetrabutylammonium tetrafluoroborate (TBABF<sub>4</sub>) in acetonitrile. The scan rate was  $200 \text{ mV s}^{-1}$ . (b) Electrochemical reduction of nitrophenyl film on SiC. The electrolyte was 0.1 M KCl in a solution of ethanol–water ( $v/v = 1:9$ ). The scan rate was  $100 \text{ mV s}^{-1}$ .

after more than 10 cycles (dashed line). The peak arises by reduction of diazonium salts cations, yielding radical species that covalently bind to the SiC surface. The decreased cathodic peak current and the negative shift of the cathodic peak potential indicate the formation of an insulating film, where either the reduction is halted or the electron transfer through the formed film is kinetically slowed down as a function of time.<sup>20</sup>

To gain insight into layer uniformity of the grafted nitrophenyl film on SiC, voltammetric response of the redox couple  $\text{Fe}(\text{CN})_6^{3-/4-}$  was measured and compared with the properties of a bare SiC electrode. On bare SiC electrodes, cyclic voltammograms show equal peak current densities of  $0.35 \pm 10 \text{ mA cm}^{-2}$ , the formal potential  $E_{1/2} = 160 \pm 10 \text{ mV}$ , and  $\Delta E = 140 \pm 20 \text{ mV}$  vs Ag/AgCl as measured at a scan rate of  $100 \text{ mV s}^{-1}$ . After modification, the  $\text{Fe}(\text{CN})_6^{3-/4-}$  response is strongly suppressed due to the insulating properties of the phenyl layer. For shorter phenyl attachment times in constant potential (e.g., at  $-0.4 \text{ V}$ ) experiments or applying less cycles, the phenyl layer become partially transparent for the redox molecules thereby generating a redox current as detected for other transducer materials like diamond in the past.<sup>20</sup>

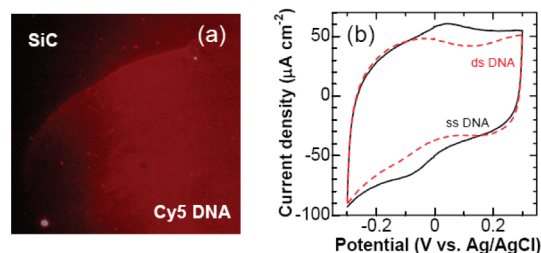
To characterize the reactivity of nitrophenyl layers on SiC surfaces, the electrochemical reaction of nitrophenyl was investigated in the aqueous solution of ethanol–water ( $v/v = 1:9$ ) with 0.1 M KCl. As shown Figure 2b, scanning from the initial potential of  $+0.7 \text{ V}$  vs Ag/AgCl, a large irreversible reduction peak appears at  $-1.10 \pm 0.05 \text{ V}$  vs Ag/AgCl. This cathodic wave is due to the electrochemical reduction of nitrophenyl ( $\text{Ar}-\text{NO}_2$ ) groups to amine- ( $\text{Ar}-\text{NH}_2$ ) or hydroxyaminophenyl ( $\text{Ar}-\text{NHOH}$ ) groups.<sup>21,22</sup> The density of nitrophenyl on the SiC surface was then evaluated using the charges transferred of the wave centered at  $-1.10 \text{ V}$  to be  $3.1 \times 10^{15} \text{ cm}^{-2}$  or  $5.1 \times 10^{-9} \text{ mol cm}^{-2}$ . In the second and subsequent cycles, the reduction peak is drastically diminished, indicating that nearly all electro-active nitrophenyl groups are reduced in the first scan. The reversible couple at  $E_{1/2} = -350 \pm 30 \text{ mV}$  vs Ag/AgCl appeared only after the first cycle, indicating an incomplete reduction process of  $-\text{Ar}-\text{NO}_2$  to  $-\text{Ar}-\text{NH}_2$ .<sup>21,22</sup> Different peak potentials of these waves from those on carbon electrodes are attributed to the features of electrodes used. As reported previously, this redox couple results from the interconversion of hydroxyaminophenyl into nitrosophenyl.<sup>21,22</sup>



**Figure 3.** XPS survey spectra of a bare SiC (a) and of a nitrophenyl-grafted SiC (b). The insert shows a detailed spectrum for the nitrogen-peak on nitrophenyl grafted SiC.

High-resolution X-ray photoelectron spectroscopy (XPS) was employed to confirm the bonding of diazonium salts to SiC. A monochromated Al K $\alpha$  beam was used as the X-ray source. The measurements were performed under 45° with respect to the surface normal. Figure 3 shows survey spectra of (a) a bare SiC and (b) of a nitrophenyl-grafted SiC. After electrochemical attachment of diazonium salts, a well-defined nitrogen emission can be detected. The absolute intensities of peaks of O(1s) at 532 eV and of C(1s) at 283 to 285 eV are enhanced, while peaks of Si(2s) at 153 eV and of Si(2p) at 101 eV decrease. A detailed spectrum of the N(1s) is shown as an inset in Figure 3 where two peaks at 399 and 406 eV are detected, the same as reported in the literature.<sup>20</sup> The N(1s) peak at 406 eV is assigned to the NO<sub>2</sub> group, and the lower binding energy N(1s) peak at 399 eV is attributed to some reduced nitrogen-containing functionalities. The storage of SiC samples in acetonitrile containing 1.0 mM of diazonium salts and 0.1 M TBABF<sub>4</sub> at room temperature without applying potentials did not generate any noncovalent adsorption of diazonium salts on the SiC surface, as confirmed by XPS measurements (not shown). These results together with electrochemical data indicate covalent bonding of nitrophenyl to the SiC surface.

The bioapplication of 3C-SiC was tested by bonding DNA to the modified surface. We applied a typical protocol to immobilized DNA.<sup>23</sup> The probe DNA was labeled with a redox center of ferrocene moiety and the cDNA was labeled with a red fluorescence marker of Cy5 dye. The fluorescence image of the bare SiC shows no emission of red light. No adsorption of DNA was found on the bare SiC surface using fluorescence measurements. After electrochemical grafting of nitrophenyl layers and the addition of DNA onto SiC surface, the functionalized area showed high intensity of red fluorescence (see Figure 4a), indicating DNA has been immobilized. As shown in Figure 4b, the voltammogram of ss DNA shows a couple of redox waves of ferrocene moiety at  $E_{1/2} = 0 \pm 5$  mV vs Ag/AgCl and  $\Delta E = 50 \pm 10$  mV, which disappear after hybridization of ss DNA with cDNA. A decrease in the redox current specifically indicates the presence of target DNA. Hybridization induces an increase in rigidity of the hybridized DNA, which inhibits the access of the terminal ferrocene moiety to the electrode surface and gives rise to a decrease of the redox current of the ferrocene moiety. This method is recognized to be a simplified detection method without labeling target DNAs or adding external electroactive species.<sup>24</sup> To the best of our knowledge, this is the first report about covalently bonding of DNA molecules on SiC surfaces. More experiments are however required to evaluate the properties



**Figure 4.** (a) Fluorescence microscopic image of a ds DNA functionalized SiC electrode and (b) cyclic voltammograms of ss DNA (solid line) and ds DNA (dashed line) modified SiC electrode in pH 7.4 phosphate buffer at a scan rate of 50 mV s<sup>-1</sup>.

of such sensors with respect to sensitivity, stability, and detection limits. Especially, to know the reaction efficiency of SiC electrodes with biomolecules such as DNA, the investigation on the linker density, the reaction conditions (such as reaction time, reaction temperature, the concentration and the amount of biomolecules, etc.) are required.

In summary, nanocrystalline 3C-SiC films are promising to be applied as electrode material for electrochemical and biochemical applications. Electrochemical grafting of SiC with diazonium salts will be a new approach for functionalization of SiC and further for covalent bonding of biomolecules like DNA, enzymes, other proteins, and antibodies. However, the dopants, doping level, surface termination, polytypes of SiC will play an important role in the electrochemical properties of SiC such as potential window, background current, and voltammetric reactivity of SiC electrodes. It should be investigated in detail before mass-production of SiC electrodes for electrochemical and biochemical experiments.

## ■ ASSOCIATED CONTENT

**S Supporting Information.** Experimental details. This material is available free of charge via the Internet at <http://pubs.acs.org>.

## ■ AUTHOR INFORMATION

### Corresponding Author

\*Phone: 0049-761-5159-647. Fax: 0049-761-5159-71647. E-mail: nianjun.yang@iaf.fraunhofer.de.

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