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Functionalization of 6H Highly Doped Silicon Carbide Surfaces for Determining Cell Electrophysiology

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Abstract:

Understanding the electrical activity of biological cells and tissue is important for medical diagnostics and bioengineering. Electrophysiology is employed to measure the electrical behavior of biological materials ranging in size from single ion channel proteins to entire organs. In order to measure the electrical properties of cells, they must be attached to a surface that is conductive and biocompatible. Silicon carbide (SiC) was used in this study because in addition to having these properties, its surface can be functionalized for protein attachment, which subsequently renders the surface amiable for cell attachment. SiC was exposed to oxygen plasma to render hydroxyl (OH) groups on its silicon (Si) face. The terminal OH groups were covalently bonded to 3-aminopropyltriethoxysilane (APTES). Raman spectroscopy measurements confirmed peaks for SiC and both oxidized and APTES functionalized SiC. The addition of APTES to SiC provided a reactive surface ready for antibody attachment and capable of supporting an antibody antigen reaction.

Introduction:

In recent years there has been an increased focus on the electrochemical properties of biological materials. Research in this field has led to significant developments in cancer, biosensor, and bioengineering research [1]. As this field of research has grown so has the need for substrates capable of greater sensitivity and selectivity. Due to its biocompatibility, electrical properties, chemical inertness, and thermal stability, SiC has proven to be an exemplary material for electrophysiological research. The process of constructing SiC-based apparatuses begins with developing an analyte-specific functionalization of SiC. In this experiment, surface chemistry of SiC was used to achieve this goal.

Experimental Procedure:

Commercially purchased 6H highly doped SiC was used in this experiment [2]. All reactions were performed on the Si face of the substrate. To begin the functionalization process, T = the samples were submerged for 5 min in trichloroethylene, succeeded by acetone, and then isopropanol. They were further cleaned using a 5:1:1 mixture of deionized water, hydrogen peroxide, and ammonium hydroxide in an 80°C environment for 10 minutes, also known as an RCA cleaning procedure.

To further remove organic contaminants and increase reactivity of the SiC surface, the substrates were then oxygen plasma cleaned using a Plasma-Therm model 790 plasma enhanced chemical vapor deposition system using a 20% oxygen/80% argon gas mixture for a one minute period [3]. This process deposited a thin oxide layer on the surface of the SiC substrates. After oxygen plasma treatment, the substrates were placed under a fume hood and exposed to air for approximately 3 h to ensure surface chemisorption of water molecules [3]. This was done to aid APTES hydrolysis in the next step of the experiment.

APTES functionalization was performed in a class 100 clean room in a nitrogen environment. The silanol-terminated SiC samples were immersed in a 49:1 volume fraction (v/v) solution of APTES in toluene for a duration of approximately 10 minutes [3]. The substrates were then dried using N_2 gas to remove any loosely attached APTES molecules from the surface [3].

Results:

Raman spectroscopy was used in order to confirm the presence of the expected functional groups after each functionalization step. The technique has not been widely used in this fashion, though its ability to determine the presence of functional groups by detecting slight shifts in laser energy caused by interactions between the incident laser and vibrational energy levels of the molecules in the sample make it a suitable and perhaps even preferable methodology for confirming the presence of functional groups.

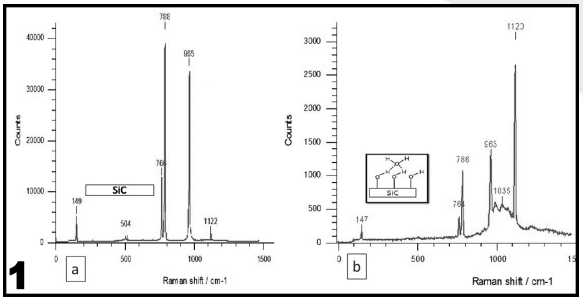

Figure 1: (a) Raman spectrum from 0 to 1500 wavenumbers of a cleaned SiC sample. (b) Raman spectrum from 0 to 1500 wavenumbers of the same sample after oxygen plasma treatment.

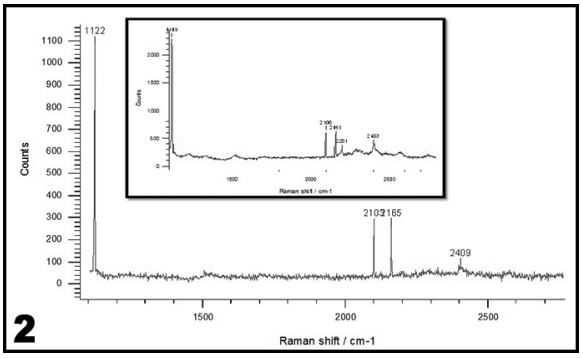

Figure 2: Raman spectrum from approximately 1100 to 2750 wavenumbers of a cleaned (refer to inset) SiC sample and the same sample after oxygen plasma treatment (larger image). A silanol terminated SiC diagram is included.

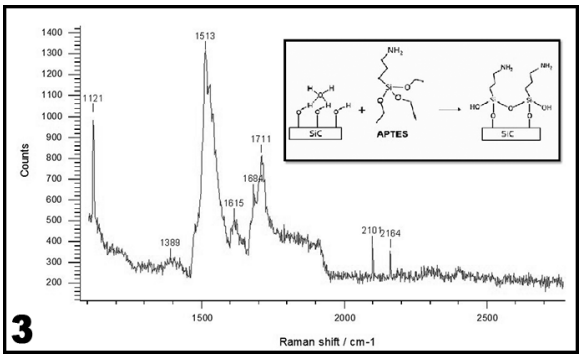

Figure 3: Raman spectrum from approximately 1100 to 2750 wavenumbers of the same sample after APTES functionalization. A diagram of the reaction is included.

In Figure 1, which displays the spectrum for the pre-oxygen treated substrate, peaks at 745 and 760 correlate to Si-C bonds, while the peak at 960 correlates to SI-Si [4]. In Figure 2, which displays the spectrum of the same sample after oxygen plasma treatment, the peak at 1120 increases significantly, indicative of the augment in Si-O bonds [4]. Figures 3 and 4 display the before and after of the APTES step, with Figure 3 corresponding to the oxygen plasma treated substrate (the inset is the cleaned substrate) and Figure 4 corresponding to the APTES functionalized surface. After the APTES functionalization, the peaks at 1620 and 1390 reciprocal centimeters indicate the presence of amines on the surface [4].

Conclusion and Future Work:

Raman spectroscopy's ability to identify the presence of added functional groups was key to the success of this project. Now that an APTES functionalized surface has been confirmed, the focus of this project will be to determine a methodology for antibody attachment, which will either be accomplished via direct attachment using a carboxyl-amine reaction involving the constant end of the antibody and the APTES surface of SiC, or via indirect attachment by utilizing intermediate layers of compounds to achieve antibody attachment. After this, the next important benchmarks will be the selective attachment of cells and the determination of their electrical properties using scanning tunneling microscopy (STM).

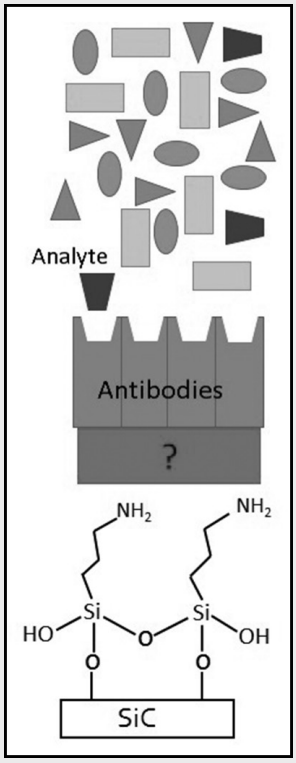

Figure 4: A schematic of the device. The region between the APTES functionalized SiC and the antibody layer depicts the uncertainty involved in how to attach the antibodies to the surface.

Acknowledgements:

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References:

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