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Single-Crystal Cubic Silicon Carbide: An *In Vivo* Biocompatible Semiconductor for Brain Machine Interface Devices

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***Abstract*—Single crystal silicon carbide (SiC) is a wide band-gap semiconductor which has shown both bio- and hemo-compatibility [1-5]. Although single crystalline SiC has appealing bio-sensing potential, the material has not been extensively characterized. Cubic silicon carbide (3C-SiC) has superior *in vitro* biocompatibility compared to its hexagonal counterparts [3, 5]. Brain machine interface (BMI) systems using implantable neuronal prosthetics offer the possibility of bi-directional signaling, which allow sensory feedback and closed loop control. Existing implantable neural interfaces have limited long-term reliability, and 3C-SiC may be a material that may improve that reliability. In the present study, we investigated *in vivo* 3C-SiC biocompatibility in the CNS of C56BL/6 mice. 3C-SiC was compared against the known immunoreactive response of silicon (Si) at 5, 10, and 35 days. The material was examined to detect CD45, a protein tyrosine phosphatase (PTP) expressed by activated microglia and macrophages. The 3C-SiC surface revealed limited immunoresponse and significantly reduced microglia compared to Si substrate.**

I. Introduction

THERE is an increasing demand for biomedical devices that interact with the cellular environment and are biocompatible for long-term implantation. The superior sensing properties of semiconductors would greatly enhance biomedical sensors, smart implants, and interfaced neural networks. Unfortunately, most semiconductors have demonstrated *in vivo* toxicity (i.e. gallium arsenide) or have demonstrated *in vivo* chemical environmental reactivity (i.e. silicon and silicates) [6-9]. Semiconductor devices made from these materials must therefore be hermetically sealed or permanently coated with other materials to remain functional and prevent chronic inflammation [10-13]. Brain machine interface (BMI) devices using implantable probes interact directly with neural networks using electrical fields via extracellular electrochemical potentials [14-17]. Due to this interaction, these micro-electrical-mechanical (MEMS) devices receive neuronal signals and also influence the extracellular ionic environment to generate action potentials [14-17].

Modern neural prosthetic devices show levels of signal degradation leading to complete device failure over a few years [10-12, 18-23]. However these devices must, by necessity, last the lifetime of the patient which can be up to over 70 years. Longevity is of paramount concern to most patients who do not desire multiple neural surgical procedures. Device failure is attributed to the acute immune response resulting from implantation-associated trauma and a chronic response resulting from contributions of material non-biocompatibility, device geometry, and material bio-fouling [10, 11, 23-27]. To date, few materials have emerged that specifically address the problem of chronic immune response while retaining satisfactory electrical performance.

Single-crystal cubic silicon carbide (3C-SiC) is a wide band gap semiconductor with vast sensing potential, is extremely resistant to physical wear and chemical corrosion, and possesses optimal tribological properties [28, 29]. The amorphous phase of this promising material has been used with appreciable success as a prosthesis coating, but this phase lacks the electrical flexibility and possesses different tribological properties compared with 3C-SiC [30-35]. Our group has undertaken a systematic study of the biocompatibility and hemocompatiblity of 3C-SiC [5]. We previously performed *in vitro* tests with immortalized and primary derived cell lines originating from skin, connective tissue, the central and peripheral nervous system, and blood platelets consistent with ISO 10993 [1-5]. Results of those experiments indicate the potential for material biocompatibility [5].

In the present study we examined the *in vivo* immune response to 3C-SiC implantation in a murine model compared to a known reactive material, silicon (Si). We found 3C-SiC to generate a minimal immune response following an extended period of implantation. Specifically, a limited activation of microglia attached to the surface of our implant. Conversely, Si produced an intense immune reaction and significant microglial activation was observed as implant-attached cells. Taken together, these results suggest 3C-SiC is a candidate material for the construction

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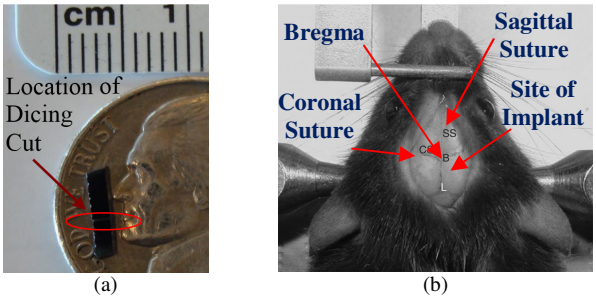
of a long-term neural implant device.

II. Experimental Methods

A. 3C-SiC/ Si (100) Implant Development

3C-SiC(001) epilayers were grown on 525 µm thick Si(001) substrates in a chemical vapor deposition (CVD) reactor [36] and diced with a diamond saw into 7 mm by 1 mm rectangles. Details concerning the samples and cleaning methods may be found in the literature [1-5]. Fig. 1(a) displays an example of the implanted samples created for this study. Importantly, samples were designed with one surface consisting of Si and the other with a 3-5 µm thick 3C-SiC film. This strategy allows for the standardization and equal comparison between materials without the caveats of material insertion protocol, acute implantation damage or brain region of implantation. In addition, an approximately 288 µm wide and 53 µm deep dicing cut was placed on the 3C-SiC surface to allow side-by-side comparison on one side of the implant.

B. In Vivo Implantation Protocol



(a) (b)

Fig. 1. (a) A digital photograph of one implant sample after removal. The 3C-SiC film is on the presented surface and the ruler and nickel are included for scale. Additional dicing cuts penetrating only the 3C-SiC film were made to allow concurrent material examination, which is seen at 3.15 mm from the inserted end. (b) A picture showing the location of the implanted 3C-SiC/ Si sample. Locations used for orientation of the surgery and measurement standards are the coronal (x axis) and sagittal (y axis) sutures as well as the bregma (origin). Image taken from [37].

The University of South Florida has an assurance #A-4100-01 on file, and animals for this experiments were maintained in accordance with the USF Institutional Animal Care and Use Committee protocol. Three C57BL/6J mice were implanted with the samples. The mice were anesthetized with isoflurane using a calibrated vaporizer and the devices were implanted at the site indicated in Fig. 1(b). The digital stereotax manipulator arm origin was set to the bregma, the sagittal suture lies on the Y axis, and the coronal suture in the X axis. A 0.75 mm wide hole was drilled in the skull starting from (2.1 mm, 0.5 mm) and extending to (2.1 mm, -0.5 mm). The sample implant was lowered into the brain to a Z-depth of -5 mm. Dental cement was used to secure the shank to the skull, and the mouse scalp was sutured closed. The mice were monitored daily for overall health and to insure proper healing of the incision. Implants were removed at 5, 10, and 35 days.

C. Fluorescence Microscopy Analysis

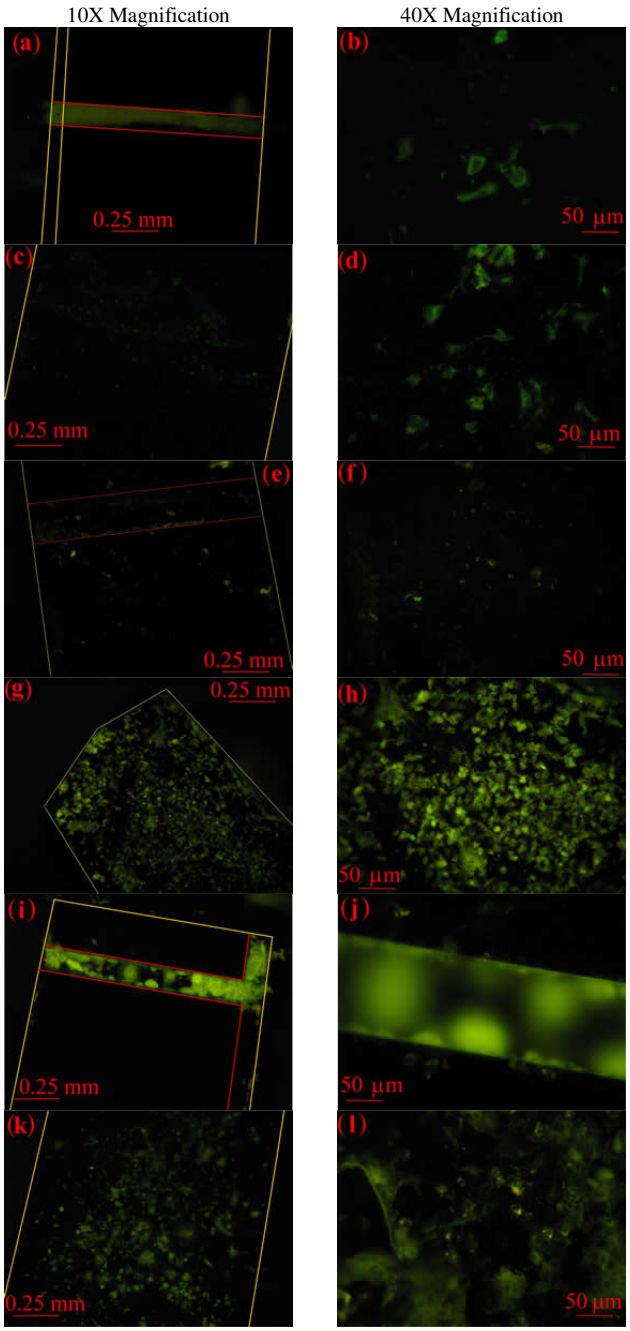


Fig. 2. Fluorescent optical micrographs indicating CD45+ immunoreactivity for the 5, 10, and 35 day implanted sample surfaces. Images (a-d) are from the 5 day, (e-h) the 10 day, and (i-l) the 35 day implant. Yellow lines delineate the sample edge, and red lines show the extent of intentional dicing cuts. (a) An image of the 3C-SiC surface showing the dicing saw cut, which exposed the underlying Si, displays increased CD45+ microglia (b) An image of the CD45+ microglia cells on the 5 day 3C-SiC surface at the shank edge near the Si interface. Both (c) and (d) indicate that the Si face shows increased CD45+ microglia. (e) The 3C-SiC face displays clusters of CD45+ microglia at the Si/ 3C-SiC interface. (f) 3C-SiC exhibits few CD45+ microglia. (g) and (h) are images indicating near encapsulation of the Si surface by CD45+ microglia. (i) The 3C-SiC surface manifests no microglial presence except near the edges of the CD45+ microglia infested dicing cuts. (j) More detailed image of the dicing cut. (k) and (l) present the surface of Si with continued encapsulation of CD45+ microglia.

To remove the implants, the mice were transcardially perfused with saline (0.9% NaCl) followed by 4% paraformaldehyde (PFA). The implants were blocked with donkey serum and sodium azide (NaN_3) in phosphate buffered saline (PBS) then sequentially incubated overnight at 4°C with 1:3,000 anti-CD45 (ab25386, AbCam, Cambridge, MA) in 1% BSA, 0.1% sodium azide, 0.2% triton, and room temperature with 1:250 Alexa Fluor® 488 donkey anti-rat IgG (H+L) (A-21208, Invitrogen, Carlsbad, CA). The samples were viewed and recorded using a Leica Microsystems DM2000 microscope with EL6000 external light source. Microscope images were taken using a Canon PowerShot S5 IS equipped with a Martin Microscope MM99 adapter.

III. Experimental Results

Immune response to 3C-SiC and Si was evaluated by direct observation of activated microglia and macrophages attached to the implant surface. CD45 is a protein marker of microglial and microphage activation [38]. Materials were removed and examined 5, 10 and 35 days after implantation.

Figs 2(a) – (d) show few CD45 positive cells on the 3C-SiC sample after 5 days. Fig. 2(a) showed the dicing cut exposed Si surface under the 3C-SiC epitaxial film generated prolific and dense CD45+ microglia. In contrast, Fig 2(b) demonstrates only six detectable microglia on the entire 3C-SiC surface. Fig. 2(c) and Fig. 2(d) showed increased presence of CD45+ microglia on the Si surface. Fig. 2(e) and 2(f) represent the 3C-SiC face after 10 days and CD45+ microglia were found clustered near the 3C-SiC/Si interface edges and within the dicing cut (Fig. 2(e)). Fig. 2(g) and Fig. 2(h) showed a nearly conformal proliferation of CD45+ microglia on the Si surface at 10 days *in vivo*.

Fig. 2(i) and Fig. 2(j) displayed the CD45+ microglia after 35 days *in vivo* on the 3C-SiC. The 3C-SiC film showed no appreciable microglial activity, except within the dicing cut, whereas the Si surface, shown in Fig. 2(k) and Fig. 2(l), displayed a continued chronic reaction from CD45+ microglia.

IV. Discussion and Conclusion

Our findings on the extent of CD45+ microglia attached to the Si surface are consistent with previous reports of Si implants into the mammalian CNS [10, 12, 26]. Increasing microglial reactivity was observed starting at day 5 until surface encapsulation at day 35, which is consistent with reported chronic reactive gliosis [10, 24, 25]. In contrast, 3C-SiC surfaces display little microglial activity 5 days after implantation. A slight increase in CD45+ microglia were present on the 3C-SiC surface by day 10, but decreased at day 35 to negligible cell counts. To insure that 3C-SiC was displaying no immunoreactivity versus a potential repulsion of microglia, a 288 µm wide and 53 µm dicing cut was put into the 3C-SiC surface to expose the underlying Si. The Si in this splice cut showed immunoreactivity equivalent to the Si side of the implant. Importantly, the lack of activated microglia in response to 3C-SiC is similar to previously reported immune interaction of parylene C and polyimide implants, considered as materials with good biocompatibility which are currently used in the neuronal environment [10, 12, 23, 39].

3C-SiC possesses desirable physical and chemical properties which make it attractive for use in implantable biomedical devices. In the present study, 3C-SiC also exhibits preferable *in vivo* qualities seen as a negligible immunoresponse to more than 30 day implantation in the CNS. The data disseminated here supports the rationale for further development of micro-implants using the 3C-SiC material. We are designing and manufacturing planar probe implants for a quantitative experiment. 3C-SiC probes will be tested against Si probes as a positive reaction control and polyimide probes as a negative control. We will have a n=6 for the time periods of 1 day, 1 week, 2 weeks, 1 month, 2 months, 4 months, and 6 months. For each period we will also use a sham experiment, or the insertion and immediate removal of the probe, to model the acute inflammatory reaction which will be compared against the results of the probes. 3C-SiC may be an ideal neural prosthetic material, and the results of this test warrant further study.

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