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A functional microarray platform with self assembled monolayers on 3C-silicon carbide.

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	0.1	0.05	0	20			0.1	0.05	0	20	
	A	B	A	B			A	B	A	B	
GLYCAN						GLYCAN					
3 1A					Lacto-N-Biose I	12A					Neocarratetraose-41, 3-di-O-sulphate (Na ⁺)
4 1B					N-Acetylglucosamine	12B					Neocarratetraose-41-O-sulphate (Na ⁺)
5 1C					β-1-4-galactosyl-galactose	12C					Neocarrahexaose-24,41, 3, 5-tetra-O-sulphate (Na ⁺)
6 1D					β-1-6 Galactosyl-N-acetyl glucosamine	12D					Neocarrahexaose-41, 3, 5-tri-O-sulphate (Na ⁺)
7 1E					β-1-3 Galactosyl-N-acetyl galactosamine	12E					Neocarrahexaose-41, 3, 5, 7-tetra-O-sulphate (Na ⁺)
8 1F					β-1-3 Gal-N-Acetyl galactosaminyl-β-1-4 Gal-β-1-4-Glc	12F					Neocarrahexaose-41, 3, 5, 7, 9-penta-O-sulphate (Na ⁺)
9 1G					Lacto-N-tetraose	12G					ΔUA-2S-GlcNS-6S
10 1H					Lacto-N-neotetraose	12H					ΔUA-GlcNS-6S
11 1I					Lacto-N-neohexaose	12I					ΔUA-2S-GlcNS
12 1J					Lacto-N-hexaose	12J					ΔUA-2S-GlcNAc-6S
13 1K					Globotriose	12K					ΔUA-GlcNAc-6S
14 1L					Tn Antigen GalNAcα1-O-Ser	12L					ΔUA-2S-GlcNAc
15 1M					Galactosyl-Tn Antigen	12M					ΔUA-GlcNAc
16 1N					α1-3 Galactobiose	12N					ΔUA-GalNAc-4S (Delta Di-4S)
17 1O					Linear B-2 Trisaccharide	12O					ΔUA-GalNAc-6S (Delta Di-6S)
18 1P					Linear B-6 Trisaccharide	12P					ΔUA-GalNAc-4S,6S (Delta Di-disE)
19 2A					α1-3,β1-4,α1-3 Galactotetraose	13A					ΔUA-2S-GalNAc-4S (Delta Di-disB)
20 2B					Galβ1-6Gal	13B					ΔUA-2S-GalNAc-6S (Delta Di-disD)
21 2C					GalNAcβ1-3Gal	13C					ΔUA-2S-GalNAc-4S-6S (Delta Di-tisS)
22 2D					GalNAcβ1-4Gal	13D					ΔUA-2S-GalNAc-6S (Delta Di-UA2S)
23 2E					Galα1-4Galβ1-4GlcNAc	13E					ΔUA-GlcNAc (Delta Di-HA)
24 2F					GalNAcα1-3Galβ1-4Glc	13F					Hyaluronan fragment (4mer)
25 2G					iso-Lacto-N-octaose (ILNO)	13G					Hyaluronan fragment (8mer)
26 2H					N,N'-Diacetyl chitobiose	13H					Hyaluronan fragment (10mer)
27 2I					N,N',N''-Triacetyl chitotriose	13I					Hyaluronan fragment (12mer)
28 2J					N,N',N'',N'''-Tetraacetyl chitotetraose	13J					Heparin
29 2K					N,N',N'',N''',N''''-Hexaacetyl chitoheptaose	13K					Chondroitin sulfate
30 2L					GlcNAcβ1-4MurNAc	13L					Dermatan sulfate
31 2M					Pentacetyl chitopentaose	13M					Chondroitin 6-sulfate
32 2N					β1-2 N-Acetylglucosamine-mannose	13N					HA - 4 10mM
33 2O					Biantennary N-linked core Pentasaccharide	13O					HA - 6 10mM
34 2P					α1-2-Mannobiose	13P					HA - 8 9.7mM
35 2Q					α1-3-Mannobiose	14B					HA-12 6.5mM
36 2R					α1-4-Mannobiose	14C					HA-14 5.6mM
37 2S					α1-6-Mannobiose	14D					HA-16 4.9mM
38 2T					α1-3,α1-6-Mannobiose	14E					HA 30000 da 2.5mg/ml
39 2U					α1-3,α1-3,α1-6-Mannopentaose	14F					HA 107000 da 2.5mg/ml
40 2V					Lacto-N-fucopentaose I	14G					HA 190000 da 2.5 mg/ml
41 2W					Lacto-N-fucopentaose II	14H					HA 220000 da 2.5 mg/ml
42 2X					Lacto-N-fucopentaose III	14I					HA 160000 da 2.5 mg/ml
43 2Y					Lacto-N-difucopentaose I	14J					Heparin sulfate 5 mg/ml
44 2Z					Lacto-N-difucopentaose II	14K					β1-3Glucan
45 3A					H-disaccharide	14L					Chondroitin disaccharide Δdi-OS, sodium salt
46 3B					2'-Fucosyllactose	17A					asialo GM2
47 3C					3'-Fucosyllactose	17B					asialo GM1
48 3D					Lewis ^x	17C					GT1c Ganglioside sugar
49 3E					Lewis ^y	17D					GT1a Ganglioside sugar
50 3F					Blood Group A Trisaccharide	17E					GD1b Ganglioside sugar
51 3G					Lactodifucotetraose (LDFT)	17F					GD1a Ganglioside sugar
52 3H					Blood Group B Trisaccharide	17G					GM1b Ganglioside sugar
53 3I					Lewis ^x	17H					GM1a Ganglioside sugar
54 3J					Blood Group H Type II Trisaccharide	17I					fucosyl GM1 Ganglioside sugar
55 3K					Lewis ^b tetrasaccharide	17J					GT2 Ganglioside sugar
56 3L					Sulpho Lewis ^x	17K					GD2 Ganglioside sugar
57 3M					Sulpho Lewis ^x	17L					GM2 Ganglioside sugar
58 3N					Monofucosyl-para-Lacto-N-hexaose IV	17M					GT3 Ganglioside sugar
59 3O					Monofucosyllacto-N-hexaose III	17N					GD3 Ganglioside sugar
60 3P					Difucosyllacto-N-hexaose	17O					GM3 Ganglioside sugar
61 3Q					Trifucosyllacto-N-hexaose	18A					LS-Tetrasaccharide d
62 3R					Lacto-N-fucopentaose VI (LNFP VI)	18B					Core type 4/Gb5 Triose structure
63 3S					Lacto-N-neodifucopentaose I (LNnDFH I)	18C					Globopentaose
64 3T					Lacto-N-neodifucopentaose II (LNnDFH II)	18E					Blood Group A Pentasaccharide
65 3U					Trifucosyllacto-N-neotetraose I (TFLNnTI)	18G					N-acetyl-D-glucosamine 6-O-sulfate sodium
66 3V					Monofucosyllacto-N-neohexaose I (MFLNnH I)	18I					D-Glucuronic acid
67 3W					Difucosyllacto-N-neohexaose I (DFLNnH I)	18J					D-Glucose-6-phosphate
68 3X					Monofucosyl(1-3)-iso-lacto-N-octaose (MFLNO)	18K					9-acetamido-9-deoxy-N-acetyl-a-D-neuraminic acid
69 3Y					Trifucosyl(1-2,1-2,1-3)-iso-lacto-N-octaose (TFILNO (1-2,1-2,1-3))	18M					4-O-(β-D-galactopyranosyl)-β-D-galactose
70 3Z					Blood Group A Tetrasaccharide	18N					6-O-(β-D-galactopyranosyl)-β-D-galactose
71 4A					Blood Group B Pentasaccharide	18O					N-glycolylneuraminic acid
72 4B					Blood Group B Tetrasaccharide	18P					4-O-(β-D-galactopyranosyl)-β-D-glucose
73 4C					Sialyl Lewis ^x	19A					Asialo galactosylated, fucosylated biantennary
74 4D					Sialyl Lewis ^x	19B					Asialo, galactosylated, tetraantennary, N-linked glycan
75 4E					Sialyllacto-N-tetraose a	19C					Monosialo(2,6), biantennary (A1)
76 4F					Monosialyl, monofucosyllacto-N-neohexose	19D					Disialo (2,6) biantennary (A2)
77 4G					Disialyl-TF	19E					Asialo, galactosylated, biantennary (NA2)
78 4H					Sialyllacto-N-fucopentaose VI (SLNFPVI)	19F					Disialo, galactosylated, fucosylated, biantennary (A2F)
79 4I					3'-Sialyllactosamine	19G					Trisialylated, galactosylated, triantennary (A3)
80 4J					6'-Sialyllactosamine	19H					Asialo, agalacto, bisected triantennary (NGA3B)
81 4K					LS-Tetrasaccharide a (LSTa)	19J					Lewis ^x Tetraose
82 4L					LS-Tetrasaccharide b (LSTb)	19K					Sialyl Lewis ^x Pentaose
83 4M					LS-Tetrasaccharide c (LSTc)	19L					Lewis ^x Pentaose
84 4N					Disialyllacto-N-tetraose	19M					Lewis ^x Tetraose
85 4O					3'-Sialyllactose	19N					Lewis ^x Pentaose
86 4P					6'-Sialyllactose	19O					Maltotriose
87 4Q					Colominic acid	19P					Maltotetraose
88 4R					Biantennary 2,6-sialylated-N-glycan-Asn

(A)Grow SiC on
both sidesOpen windows
on backside SiC

Wet etch Si

Printing

(B) **(C)**

(A)

(B)

A FUNCTIONAL MICROARRAY PLATFORM WITH SELF ASSEMBLED MONOLAYERS ON 3C-SILICON CARBIDE

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KEYWORDS: *Aminopropyltriethoxysilane, Atomic Force Microscopy, Chemical Vapor Deposition, 3(Glycidyloxypropyl)-trimethoxysilane, Glycan Arrays, Micro-Electro-Mechanical Systems, Silicon Carbide, Self-assembled Monolayers.*

ABSTRACT: Currently available bio-platforms such as microarrays and surface plasmon resonators are unable to combine high throughput multiplexing with label-free detection. As such, emerging Micro-Electro-Mechanical Systems (MEMS) and micro-plasmonics platforms offer the potential for high-resolution, high-throughput label-free sensing of biological and chemical analytes. Therefore, the search for materials capable of combining multiplexing and label free quantitation is of great significance. Recently, interest in silicon carbide (SiC) as a suitable material in numerous biomedical applications has increased due to its well explored chemical inertness, mechanical strength, bio- and hemo-compatibility and the presence of carbon that enables transfer free growth of graphene. SiC is also multifunctional as both a wide band gap semiconductor and an efficient low-loss plasmonics material, and thus is ideal for augmenting current biotransducers in biosensors. Additionally, the cubic variant, 3C-SiC, is an extremely promising material for MEMS, being a suitable platform for the easy micromachining of microcantilevers, it is capable of realizing the potential of real time miniaturized multiplexed assays. However, to realize the full potential of SiC for label free, multiplexed quantitation of biological interactions the generation of an appropriately functionalized and versatile organic monolayer suitable for the immobilization of biomolecules is critical. Herein, we address the use of various silane self-assembled monolayers (SAMs) for covalent functionalization of monocrystalline 3C-SiC films as a novel platform for the generation of functionalized microarray surfaces using high-throughput glycan arrays as the model system. We also demonstrate the ability to robotically print high throughput arrays on free standing SiC microstructures. The implementation of a SiC-based label free glycan array will provide proof-of-principle that could be extended to the immobilization of other biomolecules in a similar SiC-based array format, thus making potentially significant advances to the way biological interactions are studied.

INTRODUCTION

Microtechnologies such as Micro-Electro-Mechanical Systems (MEMS) and micro-plasmonics systems offer the potential for high-resolution, high-throughput label-free sensing of biological and chemical analytes. Both these formats have already been integrated onto lab-on-chip platforms capable of detecting antigen-antibody interactions¹⁻³, protein-protein binding⁴⁻⁵, and DNA hybridization⁶⁻⁷, with unparalleled sensitivities. We have summarized the most relevant recent studies in *Table S1 (see Supporting Information)*, however the establishment of a high throughput label-free array platform utilizing MEMS has yet to be realized. As such, fabrication of platforms capable of label-free detection of multiple target molecules in small sample volumes are of relevance for the early detection of disease. Therefore, the search for materials capable of providing these functionalities is of great significance.

Silicon carbide (SiC) is an ideal material for augmenting both MEMS and plasmonics routes due to its multifunctionality as a wide band gap (WBG) semiconductor⁸, and as an efficient low-loss plasmonics material⁹. Extensive research has demonstrated that SiC is a superior alternative to silicon (Si), due to its demonstrated bio- and hemo-compatibility¹⁰⁻¹², high Young's modulus (440 GPa), hardness (5.8 GPa) and electric breakdown field two times larger than Si¹³⁻¹⁷. Due to the chemical inertness of SiC¹⁵, it is also capable of withstanding hostile environments such as temperature and pH¹⁶⁻¹⁸. This, coupled with its double-layer capacitance and reversibility as a redox probe¹⁹, makes SiC an ideal biosensing substrate. Finally, SiC platforms allow for increased downscaling towards nanoelectromechanical systems due to the presence of carbon that can be used for growing graphene to the substrate²⁰⁻²². We have previously published a comprehensive review exploring the advances of SiC as a material system for plasmonics and MEMS²³.

MEMS and plasmonics biosensors are desirable due to their potential for label-free quantitative measurement. However,

careful attention needs to be paid to the inorganic/organic heterointerface. The generation of this organic monolayer is vital as it can directly influence the surface and interfacial energetics for both subsequent immobilization of functional biomolecules as well as the electronic²⁴ and plasmonic²⁵ properties of the heterostructure. In terms of SiC, one of the most common routes for generating inorganic/organic heterointerfaces is silanization. Alkoxy groups from organosilane molecules form self-assembled monolayers (SAMs) through surface activation of SiC via surface termination with hydroxyl groups²⁶. As such, SiC functionalization requires either hydrofluoric acid etching²⁷ or oxygen (O₂) plasma treatment to grow a thin oxide interlayer². The generation of thin oxide layers has allowed for the growth of various organosilane SAMs used in biosensing, including aminopropyltriethoxysilane (APTES) monolayers onto 3C- and 6H-SiC verified through immobilized fluorescently labelled BSA²⁸⁻²⁹, APTES functionalized 3C- and 4H-SiC nanowires verified through the biotin-streptavidin interaction^{2, 30}, and mercaptopropyltrimethoxy-silane (MPTMS) monolayers also on 4H-SiC^{15, 31}. Finally, an oligonucleotide attached to a 3(Glycidyoxypropyl)-trimethoxysilane (GOPTS) monolayer grown on Si has been fabricated; subsequent hybridization of complementary gold nanoparticle-labelled nucleic acids were detected using atomic force microscopy (AFM)³². However, the fabrication of a high-throughput label-free platform utilizing MEMS has yet to be realized.

Current established DNA³³⁻³⁴, protein³⁵⁻³⁶, glycan³⁷⁻³⁸ and lectin³⁹⁻⁴⁰ arrays allow for high-throughput multiplexing with reduced sample volume. However, without appropriate quantitative controls and complex algorithms⁴¹, results remain qualitative due to the requirement for fluorescent, photochemical or radioisotope tagging⁴². Background interference from the label itself and labelling of probe molecules for large-scale studies are also serious limitations of these platforms. Finally, the size and position of the label has been shown to induce conformational strains and steric hindrance, affecting the probes ability to interact with target structures⁴³. Hence, more sensitive and direct measurement systems are required to augment current biological platforms. Two recent studies highlight the potential of MEMS in the field of glycobiology. Gruber *et al.* (2011) developed an oligomannose cantilever sensor to detect the antiviral protein cyanovirin-N (CV-N). In this study, gold coated cantilevers were functionalized via thiol gold chemistry with trimannose; subsequent probing with CV-N was sensitive and specific to 91 pM⁴⁴. A follow up study by the same group detected *Escherichia coli* strains with distinct mannoside binding properties onto the same cantilever system as a tool for real time growth measurements⁴⁵.

Based on the assessment of the current plasmonics and MEMS biosensors, the aim of this study is to provide proof-of-principle of a high-throughput label-free platform. To achieve this, we have explored various biofunctionalizations route capable of immobilizing a variety of biomolecules using both non-contact and contact printing techniques. Here, we evaluated two organosilane SAMs formed using APTES and GOPTS to functionalize 3C-SiC via vapour-phase silanization. We show that the resulting SAMs are capable of immobilizing a functional high-throughput array of 170+ glycans, with successful immobilization determined through probing with fluorescent glycan binding proteins (or lectins) of known specificity. Finally, to test the viability of robotically arraying

samples onto microstructures, we demonstrate for the first time piezoelectric printing on our previously published free standing SiC structures⁴⁶. These results provide the critical first steps towards generating a platform that can combine established array technology with the label-free capabilities of MEMS or plasmonic systems.

MATERIALS & METHODS

3C-SiC and Surface Functionalization

All chemicals unless specified were purchased from *Sigma-Aldrich*. Throughout this study we have used thin 3C-SiC films hetero-epitaxially grown on silicon substrates. 250-nm 3C-SiC layers were grown on 150-mm Si (111) substrates in a chemical vapor furnace at 1000 °C via an alternate supply of SiH₄ and C₃H₆ as described by Wang *et al.*, (2010)⁴⁷. All fabrication steps were monitored by contact angle measurements with a Drop Shape Analyzer 25 (*Krüss*). 3C-SiC surfaces were O₂ plasma activated using a Tergeo-plus Benchtop Plasma Cleaner (*PIE Scientific*) to allow covalent functionalization of APTES and GOPTS SAMs. The chamber was first purged and then left with O₂ (50 sscm) for 10 min before plasma was ignited at 100 RF for 15 min followed by a second O₂ purge for 10 min. The samples were carefully removed and quickly sealed to prevent dust contamination. Plasma activation, through the generation of terminal hydroxyl groups, was confirmed through contact angle measurements < 10°. SiC vapour-phase silanization of GOPTS (scheme 1A) and APTES (scheme 1B) was performed in a desiccator pressurized to 25 mmHg and heated to 150 °C for 2 hr. For GOPTS functionalization 2% *N,N*-Diisopropylethylamine (DIPEA) was added to the solution. Samples were allowed to cool to room temperature and contact angles measured to confirm the growth of the monolayer. Subsequent isocyanate functionalization of the APTES monolayer was performed with varying concentrations of 1,12-dodecane diisocyanate in anhydrous dimethylformamide (DMF) for 30 min followed by washing in DMF and tetrahydrofuran (THF).

Optimized GOPTS SiC Functionalization:

A 500-nm 3C-SiC coupon was grown hetero-epitaxially on a 3×1 inch silicon substrate and plasma activation was performed, as previously described. As a revision to the desiccator, this sample was placed into a B-580 Glass Titrator Oven (*Büchi*). A glass vial with 1 mL of GOPTS and 2% DIPEA was placed next to the SiC wafer, to the back of the oven, away from the vacuum line. The oven was pressurized to 25 mmHg and heated to 150 °C to generate vapor. Vapor phase Silanization of GOPTS was left to react with the SiC surface overnight (16 hrs). Subsequently, the oven was allowed to cool back to room temperature before being vented of any left-over methoxysilane. The wafer was carefully removed and again sealed to prevent any contamination. Generation of GOPTS SAM was monitored using sample hydrophobicity contact angle measurements. AFM using a Dimension Icon AFM (*Bruker*) was performed to monitor surface chemistry generation and smoothness and roughness of the sample. Subsequent printing of glycans was also scanned to identify surface glycan density. Images were scanned in peak force tapping mode using an RTESPA-300 (*Bruker*) cantilever. The scan rate was 0.9 Hz with a resolution of 256 × 256 pixels. According to the provider, the tip radius is 8 nm. The fabrication steps were monitored using PeakForce QNM (Quantitative Nanomechanical Property Mapping) mode under air at room

temperature. The data of the images were flattened by using a 2nd order polynomial function using Nanoscope Analysis software. The root-mean-square (RMS) values were used to indicate the surface roughness of the entire image.

Glycan Array Printing and Hybridization

Glycan arrays consisting of 172 diverse glycans (*DextraLabs*) in the absence of spacers were taken from existing glycan libraries^{38-39, 48}. Glycans were amine functionalized as previously published by Day *et al.*, (2009)³⁷. Glycan arrays were fabricated as previously published by Tiralongo *et al.*, (2018)⁴⁹. In brief, glycosylamines were suspended in 1:1 DMF:Dimethyl sulfoxide (DMSO) at a concentration of 500 μ M and printed onto 3C-SiC GOPTS slides, 3C-SiC APTES slides and SuperEpoxy3 glass slides (SE3) (*ArrayIt*) using a SpotBot Extreme array spotter (*ArrayIt*) in a six-pin subarray print per glass slide format. All glycans were printed in replicates of four, including four AlexaFlour 555/647 and FITC control spots, per subarray using 946MP4 pins and a contact time of 1 second at 50% relative humidity, with pins being

reloaded after every 12 spots. The printed arrays were subsequently acetylated in 25% (v/v) acetic anhydride in methanol at 4 °C for 15 min, and then neutralized in 1:1 ethanolamine:DMF. Before hybridization, slides were blocked in 0.5 % BSA in 50 mM phosphate buffered saline (PBS), pH 7.4 for 5 min at 22 °C. After washing with PBS, each slide was dried by placing them in an empty 50 mL tube and centrifuging for 5 min at 200 $\times$ g. Quality control hybridizations were performed with sections of known glycan specificity. Subarrays were isolated using a Gene Frame and coverslip (1.5 $\times$ 1.6 cm, 65 μ L, *Abgene*) prior to the addition of the lectins. Wheat germ agglutinin (WGA, 1 μ g) prelabelled with Texas Red (TR) (*EY laboratories*) in 50 mM array PBS (PBS with 1.8 mM MgCl₂ and 1.8 mM CaCl₂), pH 7.4 containing 0.5 % BSA was allowed to hybridize for 15 min at room temperature in the dark. Following hybridization, the Gene Frame and coverslip were removed, and the slide was gently washed twice with 50 mM array PBS. Slides were dried by centrifugation for 5 min at 200 $\times$ g and allowed to air dry for a 5 min.

Scheme 1: Synthesis route for (A) GOPTS and (B) APTES SAM growth on 3C-SiC Synthesis Reagents and conditions: (A1) CVD of GOPTS: 2% N,N-Diisopropylethylamine in GOPTS, 25 mmHg, 150 °C, 2 hr/ 16 hr.; (A2) Glycan Printing: Overnight; (A3) Glycan Neutralization: Anhydrous Methanol, Acetic Anhydride, 4 °C, 15 min; (B1) CVD of APTES: APTES, 25 mmHg, 150 °C, 2 h; (B2) Isocyanate Functionalization: 8.88/17.76 % 1,12-dodecane diisocyanate in Anhydrous

dimethylformamide, RT, 30 min; (B3) Glycan Printing: 16 hr; (B4) Glycan Neutralization: Anhydrous Methanol, Acetic Anhydride, 4 °C, 15 min.

Fluorescent Image Acquisition and Data Processing

Fluorescence intensities of the array spots were measured with the ProScanArray microarray reader (*Perkin Elmer*) using a 594 nm laser set to Texas Red setting (595 nm excitation and 605 nm emission). Image analysis was carried out using the inbuilt ProScanArray imaging software, ScanArray Express (*Perkin Elmer*). Raw glycan signals were exported into Microsoft Excel 2016. The mean background was calculated from the average of DMF/DMSO blanks on the array plus three standard deviations. This was subtracted from each glycan to generate an adjusted signal. A one tailed T-test was performed with significance set at $p = 0.05$. Binding events confirmed across 3 arrays were compiled as heatmaps representing T-test and fold increases above background. 3D Measuring Laser Microscopy was performed with an OLS 5000 LEXT (*Olympus*) to characterize the silanization and printing process for the experiments performed on the single crystal SiC-on-glass and free standing SiC structures.

Fabrication of SiC-on-Glass

Single crystalline SiC with thickness of 300 nm was epitaxially grown on a Si (100) wafer using low pressure chemical vapor deposition at 1000 °C. Silane (SiH_4) and propylene (C_3H_6) were the precursors for Si and C atoms in this alternating supply epitaxy process. The wafer was then cleaned with O_2 plasma and bonded to a glass wafer (Borofloat 33 sourced from University Wafers) using a EVG520IS hot embosser. The bias voltage of anodic bonding was -1000V. The handling Si substrate of the original SiC/Si wafer was then removed using a KOH etching, leaving a transparent SiC-on-glass wafer. For detailed fabrication and characterization see ⁵⁰.

SiC free standing membrane

SiC thin films with a thickness of 300 nm were deposited on both sides of a Si wafer. A lithography process was applied to form micro windows with dimensions of $300\ \mu\text{m} \times 300\ \mu\text{m}$ using AZ5214 photoresist and MLA150 maskless aligner. Inductively Coupled Plasma (ICP) was applied to etch SiC on the bottom side, opening square windows for subsequent Si etching. The exposed Si layer was then wet-etched in KOH at 80°C until reaching the SiC film of the top side, leaving free-

standing SiC membranes. For detailed fabrication and characterization see ⁴⁶.

Printing on Microstructures

A Marathon Argus Inkjet Printer (*Arrayjet, Roslin, UK*) was used to demonstrate robotic printing onto SiC microstructures. FITC dissolved in DMF/DMSO was printed onto the SiC-on-Glass and free standing SiC membranes. Immobilization of printed spots onto microstructures was confirmed using 3D Measuring Laser Microscopy (OLS 5000 LEXT).

RESULTS AND DISCUSSION

Contact angle measurements on at least 3 random spots demonstrated SiC grown on Si had minimal asymmetry, indicating a uniform surface, (standard deviation in most cases was < 5% from the mean). Initially, an APTES SAM was grown as outlined in *Scheme 1A* to functionalize the 3C-SiC wafer for glycan deposition. *Table 1* presents the measured contact angles for each functionalized monolayer. Both surfaces functionalized with APTES, demonstrated contact angles increased by $\sim 12^\circ$. For the GOPTS monolayer, a 14° increase was observed indicating a similar increased hydrophobicity of the SiC surface. Subsequent treatment of the APTES monolayer with either 8.88% (v/v) (α -APTES) or 17.76% (v/v) (β -APTES) of 1,12-dodecane diisocyanate (*Scheme 1B*) allowed the formation of glycosyl isocyanates through the addition of a printed glycosylamine. Contact angle measurements from surface termination of isocyanate groups resulted in a 6° and 13° increase in hydrophobicity following treatment with either 8.88% (v/v) or 17.76% (v/v) of 1,12-dodecane diisocyanate, respectively.

Glycan arrays printed on the APTES monolayer generated with both concentrations of isocyanate were probed with $1\ \mu\text{g}$ WGA-TR conjugate to elucidate whether glycosylamines maintained functionality and lectin-specificity. Here, traditional fluorescent detection using a microarray scanner served as an efficient tool to compare the various functionalization routes against known protein-carbohydrate interactions. Fluorescence detection of binding events in *Figure 1* highlights the differences in signal-to-noise ratios (SNR) as measure of the relative contribution of

Table 1: Wettability of SiC surfaces as Measured via Sessile Drop Method. Monitoring of the hydrophobicity of the SiC surface before and after silanization/ isocyanate functionalization was performed using critical contact angle measurements.

Sample	Time	Measurement ($^\circ$)			Mean
		First	Second	Third	
α -APTES	Before Oxygen Plasma	63	59	59	60 ± 2.3
	After Silanization	73	72	72	72 ± 0.6
	After Isocyanate	85	75	75	78 ± 5.8
β -APTES	Before Oxygen Plasma	64	64	65	64 ± 0.6
	After Silanization	77	78	75	77 ± 1.5
	After Isocyanate	92	87	92	90 ± 2.9
α -GOPTS	Before Oxygen Plasma	60	64	57	60 ± 3.5
	After Silanization	78	74	70	74 ± 4.0
β -GOPTS	Before Oxygen Plasma	27	27	27	27 ± 0.0
	After Silanization	61	60	61	61 ± 0.6

SiC-on-glass	Before Oxygen Plasma	25	27	27	26 ± 1.2
	After Silanization	53	52	55	53 ± 1.5

Figure 1: Fluorescent Image Acquisition of SiC Glycan Array after Hybridisation with WGA-TR (Ex/Em: 595/605). (A) α -APTES; (B) β -APTES; (C) α -GOPTS; (D) β -GOPTS.

the WGA-TR binding to the noise from non-specific interactions and background autofluorescence. Figure 1A demonstrates no detectable binding to the α -APTES generated array. However, by doubling the isocyanate concentration (17.76% (v/v)) grown on the APTES monolayer, minimal binding events were visually confirmed (Figure 1B). This effect is most likely because both isocyanate groups of the 1,12-dodecane diisocyanate monomer are of equal reactivity. Both groups are capable of interacting with nearby $-NH_2$ groups of the APTES. Due to the relative length (12 carbon atoms) and symmetry, the diisocyanate is capable of copolymerizing⁵¹⁻⁵² to the APTES SAM. This cyclic structure would therefore not be able of covalently bonding to subsequent deposited glycosylamines. As such, doubling the concentration serves to pack the monomers closely together preventing looping and copolymerization, thereby increasing glycan deposition and functionality. Although promising, the maximization of glycan deposition and an increase in SNR would most likely require a further 2-fold increase in isocyanate concentration. This is not only costly, but also may result in uneven drying due to the minimal solvent volume, resulting in increased viscosity and precipitation⁵³. APTES functionalization with 1,12-dodecane diisocyanate remains a possible route for SiC

biofunctionalization, however, an alternative and more efficient method using GOPTS was explored to maximise glycan deposition and SNR.

Due to the successful growth of an APTES SAM on 3C-SiC, another methoxysilane compound, GOPTS, was investigated as a possible functionalization route. Unlike APTES, GOPTS is synthesized with an epoxide functional group and therefore does not require additional functionalization with an isocyanate. Subsequent glycan array generation on α -GOPTS followed by probing with WGA-TR demonstrated an increased SNR and glycan specificity (Figure 1C), when compared to α -APTES. This could be in part due to the discussed copolymerization of diisocyanates, or simply the increase in surface energy of GOPTS ($\sim 60 \text{ } \gamma_s/\text{mJ m}^{-2}$) when compared to APTES ($\sim 40 \text{ } \gamma_s/\text{mJ m}^{-2}$), as previously shown on glass⁵⁴. Carbohydrate immobilization on GOPTS and APTES monolayers has been previously performed on glass through liquid deposition. As with our microarrays, the authors observed a reduced signal intensity on the APTES monolayer⁵⁵. Of note, a recent paper published by Markov *et al.*, (2017) explored the use of both APTES and GOPTS as mixed monolayers to engineer properties that are optimized especially for bioelectronics applications. This work demonstrated that the

APTES-GOPTS ratio can be used to control the surface potential and subsequent cell adhesion⁵⁶.

The current GOPTS generated glycan array was compared to our in-house glycan arrays printed on SE3 slides^{39, 49} revealing a comparable glycosylamine deposition. Statistical comparison of binding and non-binding events revealed a 78.5% homology between the arrays (see Supporting Information Figure S1 and S2). These findings demonstrated a definite improvement in surface chemistry compared to APTES functionalization. Though the GOPTS arrays produced an increased SNR, they were limited when compared to the SE3 slides which showed a 1.5-fold increase in signal in some binding events. The increased relative fluorescence can be attributed to an increased density of epoxide functional groups. Additionally, the fluorescent spot diameter on each surface is significantly different. The average spot diameters for binding events on the GOPTS array were 75 μm compared to the 150 μm spot diameters of the in-house array. This confirms a decrease in glycan deposition which could also lead to the apparent reduction in SNR. To address this, the GOPTS silanization was increased from 2 hrs to overnight to maximize glycan deposition. This optimized protocol for β -GOPTS revealed a contact angle of $61^\circ \pm 0.6^\circ$ (or a 34° increase in surface hydrophobicity).

To further characterize the β -GOPTS SAM, functionalization and printing was monitored using AFM. Measurement of 5 $\mu\text{m} \times 5 \mu\text{m}$ sections indicate a 2.5 nm increase of the SiC surface maximum height (Sz: 11.6 nm) after treatment with GOPTS (Sz: 14.1 nm) (see Figure 2A/B). Additionally, the skewness and kurtosis were decreased by 0.196 and 0.78 respectively (see Supporting Information Table S2 and S3), indicating growth of a smooth monolayer. This is also apparent in the height profiles of Figure 2A/B. Of note, the roughness of the SiC surface (RMS: 1.66 nm) increased after silanization (RMS: 2.52 nm). Xing *et al.*, (2012), reported that liquid deposition of GOPTS on glass did indeed increase the roughness of the substrate but likewise also the number of adhesive cells grown on top of this monolayer⁵⁷; our results are aligned with this finding. Subsequent printing of glycans produced a dramatic maximum height increase (Sz: 253 nm). In addition, skewness (1.41) and kurtosis (3.88) measurements of the area displayed normality, thereby confirming a uniform deposition. However, as the printed spots are much larger than these areas, similar scans were performed across 50 $\mu\text{m} \times 50 \mu\text{m}$ sections (see Supporting

Information Figure S3). As with previous AFM measurements, the skewness and kurtosis was decreased and a maximum height increase of 4.9 nm was detected, confirming the uniform surface functionalization (see Supporting Information Table S4 and S5).

To image and characterize the entire glycan deposited spot, 93 $\mu\text{m} \times 93 \mu\text{m}$ sections (Figure 3) were taken, which to our best knowledge has not been previously performed on glycan microarray. As the printed geometry of microarray spots are governed by surface site density, sample viscosity, volume of deposition, temperature and humidity; here AFM allows for physical characterization of the immobilized glycan providing a more detailed examination of surface density than can be characterized through fluorescence. Of note, deposition of the glycan displays normal skewness (1.65) but is leptokurtic (9.02) indicating an annular distribution of glycan deposition. The SiC roughness after glycan printing also increased (RMS: 40.9 nm) (see Supporting Information Table S6), resulting in a significant reduced adhesion⁵⁸⁻⁵⁹. This was confirmed by measuring the adhesive contact forces between the AFM probe and the printed spot (see Supporting Information Figure S4). As these adhesive forces are nonspecific and can be directly related to the interfacial energy⁶⁰, decreased non-specific binding can be expected.

The surface area difference, across the diameter of the spot revealed a 0.11% variance, demonstrating uniform deposition of the glycan. From the height sensor profile (Figure 3C), the average roughness indicates a 32.1 nm glycan layer on the β -GOPTS functionalized SiC.

We further investigated the electrical properties of the SiC films subjected to the GOPTS silanization process. To do so, a n-type, 300-nm 3C-SiC/Si film was grown as described, and a 10 mm $\times$ 10 mm dice was acquired. Four aluminum (Al) electrodes were deposited and patterned along the edges of chips using a standard photolithography (See Supporting Information Figure S5A). These chips underwent overnight CVD with GOPTS, as described earlier, and due to the large area, monitored using 3D measuring laser microscopy. As with the previous AFM characterization, the 6 mm $\times$ 6 mm area between the Al electrodes showed an increase in roughness and also symmetry after GOPTS silanization (see Supporting Information Figure S6 and Table S7). An array of FITC spots printed in the center of the

Figure 2: 5 μm x 5 μm AFM images to characterize fabrication steps. (A) O₂ Plasma treated SiC; (B) Vapour Phase deposition of GOPTS on SiC (C) Printed glycan. Height sensor profiles are provided underneath each respective image.

Figure 3: 93 μm x 93 μm AFM image of printed glycan onto the atomically smooth 3C-SiC. (A) 2D AFM of glycan spot; (B) 3D AFM of glycan spot; (C) Height sensor profile of the microarray surface shown along the line drawn in “(A)”.

chip, indicated strong uniformity and confirmed epoxide functionality (See Supporting Information Figure S5B). The electrical conductivity of the SiC films was measured before and after the GOPTS silanization by sweeping the applied voltage from -0.5 V to 0.5 V using an Agilent B1500A semiconductor device analyzer. The voltage-current characteristics (I-V curve, solid lines) of the film exhibit strong linearity, indicating the successful Ohmic contact between Al and SiC (See Supporting Information Figure S5C). Of note, the I-V characteristics of the two different resistances (R1 and R2) can be rationalized by the contribution of carrier concentration, and/or the difference in the size of electrodes due to misalignment during the fabrication process.

Nonetheless, the experimental data shows that the I-V curve of the SiC remained unchanged after silanization, implying that the chemical treatment in our GOPTS process did not alter the electrical properties of the SiC film. This result is reasonable owing to the chemical inertness and electrical stability of the SiC material. In addition, this property can be considered a significant advantage of SiC films for mechanical resonators based biomaterial-qualification method. Here, the shift in the frequency of resonators subjected to the absorption of bio-species can be electrically measured, while the electrical noise induced from surface reaction can be minimized due to the stability of SiC. Furthermore, our recent findings⁶¹⁻⁶² in the strain sensing effect of SiC thin films combined with glycan

printing technology developed throughout this work, demonstrate the strong potential for SiC for the development of label-free biosensors based on self-sensing resonators.

Arrays generated through overnight CVD for β -GOPTS were comparable to SE3 slides demonstrating an increase in homology (82.4%) as well as comparable fold change over background (see Figure 4). Additionally, fluorescent spot diameter was increased to 150 – 200 μm indicating an increase in epoxide density on the SiC Surface. Finally decreased non-specific binding to the array surface is evident in Figure 1D, most likely due to a decrease in adhesive forces on the β -GOPTS surface when compared to α -GOPTS (Figure 1C). The differences in glycan binding by the WGA-TR conjugate on each array can be attributed to the different surface chemistries. This is most likely an effect of glycan presentation⁶³ due to the differences in the lengths of epoxide spacers used. Of note, a systematic study by Guillaume *et al.* (2005) compared different commercially available array surfaces against various fluorescent dyes, revealing that suitable combinations of surface and dye allow the generation of highly reproducible data and is critical to the reliability of the array⁶⁴. As such, the use of a different label could provide an increase in homology. Glycan density is another important factor which can affect

glycan-lectin interactions to a high extent. Yu *et al.*, (2013) demonstrated that lectin recognition of glycans can vary by 3 orders of magnitude depending on the density on the microarray⁶⁵. This is attributed to the glycan clustering effect, increasing the avidity of a lectin to a multivalent complex. Density has also been compared against antibodies present in human serum. Here, the authors noted differences in binding to both high and low densities of the given glycan⁶⁶, again highlighting the importance of multivalency.

As discussed, a pressing issue of labelling technologies is with the label itself, exacerbating non-specific binding or decreasing the binding efficiency of proteins with their target. Fei *et al.*, (2011) reported that labelling lectins with fluorescent agents can alter their binding profiles in some cases by orders of magnitude. Additionally, binding is associated with large dissociation rates that can be easily missed or deemed insignificant, as most captured lectins are washed off before detection⁶⁷. Interestingly, a paper by Nan *et al.*, (2009) derivatized GOPTS surfaces on glass with a 4-hydroxybutyric acid hydrazide linker to

Figure 4: Heatmap representation of glycan arrays probed with WGA-TR (1 µg) conjugate. Statistical significant (p= 0.05) binding events are displayed in yellow and fold changes in red (0 – 20). (A) β-GOPTS SiC Array; (B) SE3 glass

increase fluorescent intensity. In doing so, the authors were able to detect mannose structures with ConA down to 100 nM⁵⁵. Consequently, further treatment of the GOPTS surface could serve to increase the homology between the two surfaces. Though immobilization of glycans to a surface through an epoxy linkage has been shown previously^{39,68}, our array format on SiC is the first significant step to realizing a MEMS sensor

for glycan-lectin interactions. Here, our final revision of the GOPTS functionalization route provides a high density of docking sites for the specific attachment of glycans to SiC. Importantly, since biomolecules, in general, are much larger than the surface unit cell of a semiconductor, the dense GOPTS interfacial layer is necessary to passivate electron surface states and protect the SiC from solvent molecules and ions on the

organic side. Additionally, the large deposition of glycan onto the SiC surface should also minimize solvent interactions that could have a negative impact on the electronic properties of the heterostructure. Finally, the use of SiC is also significant as it has been shown that “classical” semiconductors are not ideally suited to making electronic contact to bio-organic systems due to considerable offset with respect to the HOMO-LUMO gap²⁴. Therefore, our SiC array format represents a promising alternative, being a WBG semiconductor offering advantageous electronic charge exchange with biomolecules. Our characterization by AFM shows that this method could be easily adapted onto a MEMS device to enable label free detection.

The ability to robotically print numerous samples at specific locations would also realize the potential of microcantilever arrays. These arrays, though easily fabricated through lithography, rely on individually depositing biomolecules using a micro- or nano-manipulator. This time consuming “bottleneck” is unrealistic for the generation of high-throughput MEMS. Therefore, we investigated the possibility of printing on different platforms, including single crystal SiC-on-glass. As shown in *Figure 5A*, after SiC was grown on a Si substrate, we employed anodic bonding to transfer SiC from the Si substrate onto a borofloat wafer⁶⁹. The Si layer was then completely removed using wet etching in KOH solution and RCA cleaned, forming a transparent SiC on glass platform, (*Figure 5B*). Overnight CVD of GOPTS to this SiC film for subsequent FITC printing was performed as described above. Contact angle measurements (*see Table 1*) indicated growth of a uniform monolayer with a 27 ° increase in surface hydrophobicity. *Figure 5C* shows an array of FITC spots successfully printed on the SiC-on-glass platform. It should be pointed out that, since SiC-on-glass is transparent and SiC is electrically stable

under visible wavelengths (unlike Si)⁵⁰, the possibility of printing bio-material on this new platform could enable multiple-probe measurements. For instance, the optical characterization (e.g. fluorescence) of glycan can be performed simultaneously with the electrical characterization. Finally the capability of printing on SiC MEMS structures was tested on SiC membranes, which have been fabricated previously⁴⁶ using the lithography process shown in *Figure 6A*. Here, SiC thin films are deposited on both sides of a Si wafer and then the SiC layer at the bottom was etched using Inductive Coupled Plasma (ICP) to open square windows. These bottom-side SiC windows were utilized as a hard-mask for wet-etching the SiC substrate allowing the generation of a free standing SiC membrane measuring 300 μm × 300 μm × 0.3 μm, as shown in *Figure 6B*. We applied the burst test to the as-fabricated SiC membrane, by mounting the SiC membrane on an enclosed air-cavity that was connected to a pressure controller. The experimental data showed excellent mechanical strength of the 300 nm thin SiC membranes, which can withstand pressure of at least 2 Bar. This robustness enables direct printing of materials onto suspended SiC MEMS structures. A Marathon Argus Inkjet Printer (*ArrayJet, Roslin, UK*) was used to print onto the free standing SiC area (300 μm × 300 μm). Subsequent 3D measuring laser microscopy images (*Figure 6C*) indicate good adhesion between printed FITC spot and the suspended SiC surface. Of note, a recent paper by Da Silva *et al.* (2017) demonstrated that GOPTS treatment of free standing silk fibroin films decreased cytotoxicity against osteoblast cells⁷⁰. Based on this, GOPTS treatment of our free standing SiC microstructures potentially represents an efficient platform for cell growth and subsequent biotesting through array printing.

Figure 5: (A) Fabrication process of single crystal SiC bonded onto glass substrate. (B) Photograph of a transparent 3C-SiC-on-glass chip formed using wafer bonding. (C) 50x Laser Intensity Scan for 3D Volume analysis of 9 FITC spots printed on SiC-on-glass. The scale to the right is a Box and Whisker plot indicating volume (μm³) of the 64 FITC printed spots there is an 8.55% variance within this subset.

Figure 6: (A) Fabrication process of free standing SiC membranes. (B) An SEM image of a SiC nanomembrane which a FITC spot was printed on. (C) 10 $\times$ Laser Scan for 3D Height analysis of FITC spot printed on free standing SiC membrane

To the best of our knowledge, this is the first attempt to demonstrate printing biomaterials on free standing SiC structures. Successful immobilization onto free standing SiC microstructures is vital for the development of SiC MEMS devices that are capable of real time biological detection and quantification to appropriately determine the binding affinity profiles of non-labelled probes. Validation of our SiC surface using an array format for both printing and probing is a critical step in the development of similar MEMS that combines multiplexing and label-free detection of glycans.

CONCLUSION

Previous research has highlighted the need to both understand growth of SAMs capable of generating high-throughput array platforms and their ability to be integrated onto semiconducting surfaces to create label-free detection technologies. To realize this first step, we have demonstrated successful covalent functionalization of epitaxial 3C-SiC films on silicon for glycans. Two organosilanes, APTES and GOPTS were compared as biofunctionalization routes for SiC MEMS. Contact angle measurements indicated good uniformity through vapour-phase silanization. These SAMs were biofunctionalized with glycans that could be covalently bonded to the substrate surfaces. Additionally, in depth AFM characterisation of the GOPTS microarrays revealed strong epoxide surface density. This was verified through specific lectin-glycan interactions and found to be in good accordance with our in-house glycan array published previously. Finally, we have demonstrated the capability to robotically print onto SiC functionalized on free standing SiC micro-structures. These results have provided important characterisation for our SiC functionalization route, providing vital framework for any future work to be completed on a MEMS platform. Due to the epoxy functional group from our high quality GOPTS monolayers it is possible to conjugate a wide variety of molecules including proteins, glycans and DNA. Here, we have shown successful immobilization of

biologically functional glycans onto a 3C-SiC monolayer, thus serving as proof-of-principle for the fabrication of subsequent label-free MEMS or plasmonics systems.

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AUTHOR CONTRIBUTIONS

The manuscript was written through contributions of all authors. OC wrote the initial draft of the paper, performed all the experiments, data analysis and generated the Figures. HPP and NTN provided the samples for electrical measurements, SiC-on-glass and free standing SiC membranes, as well as contributing to the writing and Figures associated with these experiments. BW devised the initial desiccator set up for the APTES silanization. SL was instrumental in the generation of the AFM data. JT conceived, designed, coordinated and revised the paper. JT planned together with OC all experiments, and CJD was involved in the glycan array printing and data analysis aspect of the paper. All authors have given approval to the final version of the manuscript.

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ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Supporting Information for Publication (PDF)

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ABBREVIATIONS

AFM; Atomic Force Microscopy, APTES; aminopropyltriethoxysilane, CVD; Chemical Vapor Deposition, GOPTS; 3 (Glycidyloxypropyl)-trimethoxysilane, MEMS; Microelectromechanical systems, SAM; Self-assembled monolayer, SiC; Silicon Carbide.

REFERENCES

1. Hwang, K. S.; Lee, J. H.; Park, J.; Yoon, D. S.; Park, J. H.; Kim, T. S., In-situ quantitative analysis of a prostate-specific antigen (PSA) using a nanomechanical PZT cantilever. *Lab Chip* **2004**, *4* (6), 547-52.
2. Williams, E.; Davydov, A.; Motayed, A.; Sundaresan, S.; Bocchini, P.; Richter, L.; Stan, G.; Steffens, K.; Zangmeister, R.; Schreifels, J.; V. Rao, M., *Immobilization of streptavidin on 4H-SiC for biosensor development*. 2012; Vol. 258, p 6056-6063.
3. Arntz, Y.; Seelig, J. D.; Lang, H. P.; Zhang, J.; Hunziker, P.; Ramseyer, J. P.; Meyer, E.; Hegner, M.; Gerber, C., Label-free protein assay based on a nanomechanical cantilever array. *Nanotechnology* **2003**, *14* (1), 86-90.
4. Nugaeva, N.; Gfeller, K. Y.; Backmann, N.; Lang, H. P.; Duggelin, M.; Hegner, M., Micromechanical cantilever array sensors for selective fungal immobilization and fast growth detection. *Biosens Bioelectron* **2005**, *21* (6), 849-56.
5. Lee, J. H.; Yoon, K. H.; Hwang, K. S.; Park, J.; Ahn, S.; Kim, T. S., Label free novel electrical detection using micromachined PZT monolithic thin film cantilever for the detection of C-reactive protein. *Biosens Bioelectron* **2004**, *20* (2), 269-75.
6. Mukhopadhyay, R.; Lorentzen, M.; Kjems, J.; Besenbacher, F., Nanomechanical sensing of DNA sequences using piezoresistive cantilevers. *Langmuir* **2005**, *21* (18), 8400-8.
7. Hansen, K. M.; Ji, H. F.; Wu, G.; Datar, R.; Cote, R.; Majumdar, A.; Thundat, T., Cantilever-based optical deflection assay for discrimination of DNA single-nucleotide mismatches. *Anal Chem* **2001**, *73* (7), 1567-71.
8. Harris, G. L., *Properties of Silicon Carbide*. INSPEC: London, 1995; p 1-297.
9. Boltasseva, A.; Atwater, H. A., Materials science. Low-loss plasmonic metamaterials. *Science* **2011**, *331* (6015), 290-1.
10. Frewin, C. L.; Locke, C.; Sadow, S. E.; Weeber, E. J. In *Single-crystal cubic silicon carbide: An in vivo biocompatible semiconductor for brain machine interface devices*, 2011 Annual International Conference of the IEEE Engineering in Medicine and Biology Society, Aug. 30 2011-Sept. 3 2011; 2011; pp 2957-2960.
11. Sadow, S. E.; Coletti, C.; Frewin, C.; Schettini, N. C.; Oliveros, A.; Jaroszeski, M., Single-crystal Silicon Carbide: A Biocompatible and Hemocompatible Semiconductor for Advanced Biomedical Applications. *MRS Proceedings* **2011**, *1246*, 1246-B08-08.
12. Sadow, S., *Silicon Carbide Biotechnology*. Elsevier: 2016; p 378.
13. Madou, M. J.; Madou, M. J., *Fundamentals of Microfabrication*. CRC Press: 2002; p 752.
14. Li, X.; Wang, X.; Bondokov, R.; Morris, J.; An, Y. H.; Sudarshan, T. S., Micro/nanoscale mechanical and tribological characterization of SiC for orthopedic applications. *J Biomed Mater Res B Appl Biomater* **2005**, *72* (2), 353-61.
15. Petoral, R. M.; Yazdi, G. R.; Lloyd Spetz, A.; Yakimova, R.; Uvdal, K., Organosilane-functionalized wide band gap semiconductor surfaces. *Applied Physics Letters* **2007**, *90* (22), 223904.
16. Coletti, C.; Frewin, C. L.; Sadow, S. E.; Hetzel, M.; Virojanadara, C.; Starke, U., Surface studies of hydrogen etched 3C-SiC(001) on Si(001). *Applied Physics Letters* **2007**, *91* (6), 061914.
17. Dhar, S.; Seitz, O.; Halls, M. D.; Choi, S.; Chabal, Y. J.; Feldman, L. C., Chemical properties of oxidized silicon carbide surfaces upon etching in hydrofluoric acid. *J Am Chem Soc* **2009**, *131* (46), 16808-13.
18. Zhang, Z.; Li, L.; Xie, X.; Xiao, D.; He, W., Optimization Design and Research Character of the Passive Electric Field Sensor. *IEEE Sensors Journal* **2014**, *14* (2), 508-513.
19. Zhuang, H.; Yang, N.; Zhang, L.; Fuchs, R.; Jiang, X., Electrochemical properties and applications of nanocrystalline, microcrystalline, and epitaxial cubic silicon carbide films. *ACS Appl Mater Interfaces* **2015**, *7* (20), 10886-95.
20. Benjamin, V. C.; Mohsin, A.; Neeraj, M.; Atieh Ranjbar, K.; Barry, W.; Francesca, I., Graphitized silicon carbide microbeams: wafer-level, self-aligned graphene on silicon wafers. *Nanotechnology* **2014**, *25* (32), 325301.
21. Aristov, V. Y.; Urbanik, G.; Kummer, K.; Vyalikh, D. V.; Molodtsova, O. V.; Preobrajenski, A. B.; Zakharov, A. A.; Hess, C.; Hanke, T.; Buchner, B.; Vobornik, I.; Fujii, J.; Panaccione, G.; Ossipyan, Y. A.; Knupfer, M., Graphene synthesis on cubic SiC/Si wafers. perspectives for mass production of graphene-based electronic devices. *Nano Lett* **2010**, *10* (3), 992-5.
22. Juang, Z.-Y.; Wu, C.-Y.; Lo, C.-W.; Chen, W.-Y.; Huang, C.-F.; Hwang, J.-C.; Chen, F.-R.; Leou, K. C.; Tsai, C.-H., *Synthesis of graphene on silicon carbide substrates at low temperature*. 2009; Vol. 47, p 2026-2031.
23. Cooper, O.; Wang, B.; Brown, C. L.; Tiralongo, J.; Iacopi, F., Toward Label-Free Biosensing With Silicon Carbide: A Review. *IEEE Access* **2016**, *4*, 477-497.
24. Stutzmann, M.; Garrido, J. A.; Eickhoff, M.; Brandt, M. S., Direct biofunctionalization of semiconductors: A survey. *physica status solidi (a)* **2006**, *203* (14), 3424-3437.
25. Spackova, B.; Wrobel, P.; Bockova, M.; Homola, J., Optical Biosensors Based on Plasmonic Nanostructures: A Review. *Proceedings of the IEEE* **2016**, *104* (12), 2380-2408.
26. Ulman, A., *Ultrathin organic films*. Academic Press: Boston, 1991.
27. Starke, U.; Bram, C.; Steiner, P. R.; Hartner, W.; Hammer, L.; Heinz, K.; Müller, K., The (0001)-surface of 6H-SiC: morphology, composition and structure. *Applied Surface Science* **1995**, *89* (2), 175-185.
28. Schoell, S. J.; Hoeb, M.; Sharp, I. D.; Steins, W.; Eickhoff, M.; Stutzmann, M.; Brandt, M. S., Functionalization of 6H-SiC surfaces with organosilanes. *Applied Physics Letters* **2008**, *92* (15), 153301.
29. Schoell, S. J.; Sachsenhauser, M.; Oliveros, A.; Howgate, J.; Stutzmann, M.; Brandt, M. S.; Frewin, C. L.; Sadow, S. E.; Sharp, I. D., Organic functionalization of 3C-SiC surfaces. *ACS Appl Mater Interfaces* **2013**, *5* (4), 1393-9.
30. Williams, E. H.; Schreifels, J. A.; Rao, M. V.; Davydov, A. V.; Oleshko, V. P.; Lin, N. J.; Steffens, K. L.; Krylyuk, S.; Bertness, K. A.; Manocchi, A. K.; Koshka, Y., Selective streptavidin bioconjugation on silicon and silicon carbide nanowires for biosensor applications. *Journal of Materials Research* **2012**, *28* (1), 68-77.

31. Yakimova, R.; Petoral, R. M.; Yazdi, G. R.; Vahlberg, C.; Lloyd Spetz, A.; Uvdal, K., Surface functionalization and biomedical applications based on SiC. *Journal of Physics D: Applied Physics* **2007**, *40* (20), 6435-6442.
32. Dubrovina, E. V.; Presnova, G. V.; Rubtsova, M. Y.; Egorov, A. M.; Grigorenko, V. G.; Yaminsky, I. V., The Use of Atomic Force Microscopy for 3D Analysis of Nucleic Acid Hybridization on Microarrays. *Acta Naturae* **2015**, *7* (2), 108-114.
33. Kemp, J.; Davis, R.; White, L.; Wang, X.; Webb, C., A novel method for STR-based DNA profiling using microarrays. *2005*, Vol. 50, p 1109-13.
34. Trevino, V.; Falciani, F.; Barrera-Saldana, H. A., DNA microarrays: a powerful genomic tool for biomedical and clinical research. *Mol Med* **2007**, *13* (9-10), 527-41.
35. Pawletz, C. P.; Charboneau, L.; Bichsel, V. E.; Simone, N. L.; Chen, T.; Gillespie, J. W.; Emmert-Buck, M. R.; Roth, M. J.; Petricoin, I. E.; Liotta, L. A., Reverse phase protein microarrays which capture disease progression show activation of pro-survival pathways at the cancer invasion front. *Oncogene* **2001**, *20* (16), 1981-9.
36. Nishizuka, S.; Charboneau, L.; Young, L.; Major, S.; Reinhold, W. C.; Waltham, M.; Kourou-Mehr, H.; Bussey, K. J.; Lee, J. K.; Espina, V.; Munson, P. J.; Petricoin, E., 3rd; Liotta, L. A.; Weinstein, J. N., Proteomic profiling of the NCI-60 cancer cell lines using new high-density reverse-phase lysate microarrays. *Proc Natl Acad Sci U S A* **2003**, *100* (24), 14229-34.
37. Day, C. J.; Tiralongo, J.; Hartnell, R. D.; Logue, C. A.; Wilson, J. C.; von Itzstein, M.; Korolik, V., Differential carbohydrate recognition by *Campylobacter jejuni* strain 11168: influences of temperature and growth conditions. *PLoS One* **2009**, *4* (3), e4927.
38. Blixt, O.; Head, S.; Mondala, T.; Scanlan, C.; Huflejt, M. E.; Alvarez, R.; Bryan, M. C.; Fazio, F.; Calarese, D.; Stevens, J.; Razi, N.; Stevens, D. J.; Skehel, J. J.; van Die, I.; Burton, D. R.; Wilson, I. A.; Cummings, R.; Bovin, N.; Wong, C. H.; Paulson, J. C., Printed covalent glycan array for ligand profiling of diverse glycan binding proteins. *Proc Natl Acad Sci U S A* **2004**, *101* (49), 17033-8.
39. Arndt, N. X.; Tiralongo, J.; Madge, P. D.; von Itzstein, M.; Day, C. J., Differential carbohydrate binding and cell surface glycosylation of human cancer cell lines. *J Cell Biochem* **2011**, *112* (9), 2230-40.
40. Tao, S. C.; Li, Y.; Zhou, J.; Qian, J.; Schnaar, R. L.; Zhang, Y.; Goldstein, I. J.; Zhu, H.; Schneck, J. P., Lectin microarrays identify cell-specific and functionally significant cell surface glycan markers. *Glycobiology* **2008**, *18* (10), 761-9.
41. Akbani, R.; Becker, K. F.; Carragher, N.; Goldstein, T.; de Koning, L.; Korf, U.; Liotta, L.; Mills, G. B.; Nishizuka, S. S.; Pawlak, M.; Petricoin, E. F., 3rd; Pollard, H. B.; Serrels, B.; Zhu, J., Realizing the promise of reverse phase protein arrays for clinical, translational, and basic research: a workshop report: the RPPA (Reverse Phase Protein Array) society. *Mol Cell Proteomics* **2014**, *13* (7), 1625-43.
42. Hall, D. A.; Ptacek, J.; Snyder, M., Protein microarray technology. *Mech Ageing Dev* **2007**, *128* (1), 161-7.
43. Ramachandran, N.; Larson, D. N.; Stark, P. R.; Hainsworth, E.; LaBaer, J., Emerging tools for real-time label-free detection of interactions on functional protein microarrays. *FEBS J* **2005**, *272* (21), 5412-25.
44. Gruber, K.; Horlacher, T.; Castelli, R.; Mader, A.; Seeberger, P. H.; Hermann, B. A., Cantilever array sensors detect specific carbohydrate-protein interactions with picomolar sensitivity. *ACS Nano* **2011**, *5* (5), 3670-8.
45. Mader, A.; Gruber, K.; Castelli, R.; Hermann, B. A.; Seeberger, P. H.; Radler, J. O.; Leisner, M., Discrimination of *Escherichia coli* strains using glycan cantilever array sensors. *Nano Lett* **2012**, *12* (1), 420-3.
46. Nguyen, T. K.; Phan, H. P.; Kamble, H.; Vadivelu, R.; Dinh, T.; Iacopi, A.; Walker, G.; Hold, L.; Nguyen, N. T.; Dao, D. V., Superior Robust Ultrathin Single-Crystalline Silicon Carbide Membrane as a Versatile Platform for Biological Applications. *ACS Appl Mater Interfaces* **2017**, *9* (48), 41641-41647.
47. Wang, L.; Dimitrijevic, S.; Han, J.; Iacopi, A.; Hold, L.; Tanner, P.; Harrison, H. B., Growth of 3C-SiC on 150-mm Si(100) substrates by alternating supply epitaxy at 1000°C. *Thin Solid Films* **2011**, *519* (19), 6443-6446.
48. Huflejt, M. E.; Vuskovic, M.; Vasiliu, D.; Xu, H.; Obukhova, P.; Shilova, N.; Tuzikov, A.; Galanina, O.; Arun, B.; Lu, K.; Bovin, N., Anti-carbohydrate antibodies of normal sera: findings, surprises and challenges. *Mol Immunol* **2009**, *46* (15), 3037-49.
49. Tiralongo, J.; Cooper, O.; Litfin, T.; Yang, Y.; King, R.; Zhan, J.; Zhao, H.; Bovin, N.; Day, C. J.; Zhou, Y., YesU from *Bacillus subtilis* preferentially binds fucosylated glycans. *Sci Rep* **2018**, *8* (1), 13139.
50. Phan, H. P.; Cheng, H. H.; Dinh, T.; Wood, B.; Nguyen, T. K.; Mu, F.; Kamble, H.; Vadivelu, R.; Walker, G.; Hold, L.; Iacopi, A.; Haylock, B.; Dao, D. V.; Lobino, M.; Suga, T.; Nguyen, N. T., Single-Crystalline 3C-SiC anodically Bonded onto Glass: An Excellent Platform for High-Temperature Electronics and Bioapplications. *ACS Appl Mater Interfaces* **2017**, *9* (33), 27365-27371.
51. Lyman, D. J., Polyurethanes. I. The solution polymerization of diisocyanates with ethylene glycol. *Journal of Polymer Science* **1960**, *45* (145), 49-59.
52. Lu, Q.-W.; Hoyer, T. R.; Macosko, C. W., Reactivity of common functional groups with urethanes: Models for reactive compatibilization of thermoplastic polyurethane blends. *Journal of Polymer Science Part A: Polymer Chemistry* **2002**, *40* (14), 2310-2328.
53. Berger, M. N.; Tidswell, B. M., Dilute solution viscosity and conformation of poly-n-hexyl isocyanate. *Journal of Polymer Science: Polymer Symposia* **2007**, *42* (3), 1063-1075.
54. Lamprou, D.; Smith, J.; Nevell, T.; Barbu, E.; Willis, C.; Tsibouklis, J., Towards the Determination of Surface Energy at the Nanoscale: A Further Assessment of the AFM-Based Approach. 2010; Vol. 5, p 137.
55. Nan, G.; Yan, H.; Yang, G.; Jian, Q.; Chen, C.; Li, Z., The hydroxyl-modified surfaces on glass support for fabrication of carbohydrate microarrays. *Curr Pharm Biotechnol* **2009**, *10* (1), 138-46.
56. Markov, A.; Wolf, N.; Yuan, X.; Mayer, D.; Maybeck, V.; Offenhausser, A.; Wordenweber, R., Controlled Engineering of Oxide Surfaces for Bioelectronics Applications Using Organic Mixed Monolayers. *ACS Appl Mater Interfaces* **2017**, *9* (34), 29265-29272.
57. Xing, G. W.; Xiang, S.; Xue, W.; Aodeng, G. W.; Liu, Y.; Zhang, J. H.; Lin, J. M., Capture of cervical exfoliative cells on a glass slide coated by 3-glycidyloxypropyl trimethoxysilane and poly-L-lysine. *J Pharm Anal* **2012**, *2* (3), 174-179.
58. Ferri, F. A.; Malegori, G.; Enachescu, M.; Veis, M.; Behary, N.; Das, P. K.; De Oliveira, R. R. L.; Horng, T. L.; Bellitto, V. J.; Gupta, V., *Measurement of the Nanoscale Roughness by Atomic Force Microscopy: Basic Principles and Applications*. INTECH Open Access Publisher: 2012.
59. Liu, D. L.; Martin, J.; Burnham, N. A., Optimal roughness for minimal adhesion. *Applied Physics Letters* **2007**, *91* (4), 043107.
60. Creuzet, F.; Ryschenkow, G.; Arribart, H., A New Tool for Adhesion Science: The Atomic Force Microscope. *The Journal of Adhesion* **2006**, *40* (1), 15-25.
61. Phan, H.-P.; Dinh, T.; Kozeki, T.; Nguyen, T.-K.; Qamar, A.; Namazu, T.; Nguyen, N.-T.; Dao, D. V., Nano strain-amplifier: Making ultra-sensitive piezoresistance in nanowires possible without the need of quantum and surface charge effects. *Applied Physics Letters* **2016**, *109* (12), 123502.
62. Phan, H.-P.; Qamar, A.; Dao, D. V.; Dinh, T.; Wang, L.; Han, J.; Tanner, P.; Dimitrijevic, S.; Nguyen, N.-T., Orientation dependence of the pseudo-Hall effect in p-type 3C-SiC four-terminal devices under mechanical stress. *RSC Advances* **2015**, *5* (69), 56377-56381.
63. Grant, O. C.; Smith, H. M.; Firsova, D.; Fadda, E.; Woods, R. J., Presentation, presentation, presentation! Molecular-level insight into linker effects on glycan array screening data. *Glycobiology* **2014**, *24* (1), 17-25.
64. Guillaume, B.; Buness, A.; Schmidt, C.; Klimek, F.; Moldenhauer, G.; Huber, W.; Arlt, D.; Korf, U.; Wiemann, S.; Poustka, A., Systematic comparison of surface coatings for protein microarrays. *Proteomics* **2005**, *5* (18), 4705-12.

65. Yu, K.; Creagh, A. L.; Haynes, C. A.; Kizhakkedathu, J. N., Lectin interactions on surface-grafted glycostructures: influence of the spatial distribution of carbohydrates on the binding kinetics and rupture forces. *Anal Chem* **2013**, *85* (16), 7786-93.
66. Oyelaran, O.; McShane, L. M.; Dodd, L.; Gildersleeve, J. C., Profiling human serum antibodies with a carbohydrate antigen microarray. *J Proteome Res* **2009**, *8* (9), 4301-10.
67. Fei, Y.; Sun, Y. S.; Li, Y.; Lau, K.; Yu, H.; Chokhawala, H. A.; Huang, S.; Landry, J. P.; Chen, X.; Zhu, X., Fluorescent labeling agents change binding profiles of glycan-binding proteins. *Mol Biosyst* **2011**, *7* (12), 3343-52.
68. Day, C. J.; Tram, G.; Hartley-Tassell, L. E.; Tiralongo, J.; Korolik, V., Assessment of glycan interactions of clinical and avian isolates of *Campylobacter jejuni*. *BMC Microbiol* **2013**, *13* (1), 228.
69. Dinh, T.; Phan, H.-P.; Kashaninejad, N.; Nguyen, T.-K.; Dao, D. V.; Nguyen, N.-T., An On-Chip SiC MEMS Device with Integrated Heating, Sensing, and Microfluidic Cooling Systems. *Advanced Materials Interfaces* **2018**, *5* (20), 1800764.
70. da Silva, R. R.; Cavicchioli, M.; Lima, L. R.; Otoni, C. G.; Barud, H. S.; Santagneli, S. H.; Tercjak, A.; Amaral, A. C.; Carvalho, R. A.; Ribeiro, S. J. L., Fabrication of Biocompatible, Functional, and Transparent Hybrid Films Based on Silk Fibroin and Epoxy Silane for Biophotonics. *ACS Appl Mater Interfaces* **2017**, *9* (33), 27905-27917.

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