Silicon Carbide membranes as substrate for Synchrotron measurements

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Silicon Nitride (SiN) membranes have long been the substrate of choice for many different synchrotron techniques at very different wavelengths (from IR to hard x-rays), due to their ease of production, relative robustness even in films <200 nm in thickness, and compatibility with standard microfabrication techniques. Here we present a set of data referring to custom-made Silicon Carbide (SiC) windows. We measured SiC surface roughness, mechanical robustness and membrane transmission both at IR and soft x-rays wavelengths, and compared the data with standard Si₃N₄, acquired in the same conditions. Further, we grew HEK293T cells both on Si₃N₄ and SiC membranes, and analysed them with IR and soft X-ray microscopy. Our data demonstrates how SiC is an excellent choice as membrane material for synchrotron measurements, since it shows higher transmission and higher robustness as compared to Si₃N₄ of the same thickness, and an improved compatibility for cell culturing, allowing to postulate their use also for bio-oriented research.

KEYWORDS: Silicon Carbide; X-ray fluorescence; InfraRed Spectroscopy; X-ray Microscopy; Cell culturing.

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1. Introduction

Silicon nitride membranes have been one of the supports of choice for synchrotron measurements for three decades [1]. This is due to the ease of deposition of low stress silicon nitride films via Low Pressure Chemical Vapour Deposition (LPCVD) on silicon substrates. These in turn can be patterned with a standard and readily available combination of photolithography, dry and wet etching. Typical commercial material comes with stress < 250 MPa, allowing membranes as thin as 10 nm and as large as several millimetres [2]. Since the material is nearly chemically inert, relatively cheap, mechanically robust, and compatible with both lithographic and chemical/biological processes, it is pretty much the substrate of choice for many imaging and spectroscopy tools requiring a high degree of substrate transmission over a wide wavelengths range, from infrared to X-ray [1,3-6], especially with biological samples.

Some of the drawbacks are the relatively poor transmission of silicon nitride in the IR, where its spectrum overlaps with the vibrational modes of nucleic acids and carbohydrates, and of course the presence of nitrogen, which does not allow spectroscopy, photoemission, or fluorescence of this element.

The fundamental motivations leading to test silicon carbide as a possible candidate for synchrotron measurements were the ability to study nitrogen photoemission and fluorescence, coupled with the push of having samples mounted on substrates that are compatible with a number of spectroscopic and imaging techniques, while retaining, and possibly improving, a high photon transmission at the working energies.

The objectives of the present work are as follows: the deposited silicon carbide must have a low enough residual stress to withstand the membrane fabrication process and normal usage. As a corollary, it must be compatible with standard microfabrication techniques and tools. The substrate must be transparent to IR and x-rays, as well as

being able to withstand a high-vacuum to atmosphere pressure differential. Finally, it must be compatible with cell growth protocols.

2. Experimental work

2.1 Silicon Carbide growth and patterning

Epitaxial 3C SiC films were deposited at 1000°C in an EpiFlx reactor, designed and built by SPT Microtechnology Inc in collaboration with Griffith University. This 300mm compatible large batch LPCVD SiC reactor provides highly uniform deposition with a void-free SiC/Si interface. The growth was performed on 150 mm diameter on both the sides of Double Side Polished, <100>, p-doped, 400µm thick silicon wafers at the Queensland Microtechnology facility, at Griffith University, Nathan (QLD), Australia. The proprietary epitaxial process allows growing low stress, thin SiC layers on silicon wafers. Further, double sided deposition eliminates wafer bow as a result of the tensile SiC film and enables SiC to be used as a mask for the subsequent through Si etch. The carbide was grown at several thicknesses, namely 17, 50, 100, 200 and 400 nm. Membranes were fabricated from the double sided SiC coated wafers following deposition, using standard photolithography techniques. Plasma etching of the SiC was performed in an STS Inductively Coupled Plasma (ICP) etch system using HCl gas. The remaining photo resist was removed in a pure O₂ plasma in a Tegal 905 plasma asher. Through Si etch was achieved using 30% KOH at 80°C. An RCA 2 clean (6: H₂O, 1:HCl and 1: H₂O₂ @ 70°C) was then used to remove any metal ion contamination. Each window was separated by snapping along defined etch pits.

2.2 Mechanical strength tests and AFM measurements

The SiC membranes were then tested for porosity, mechanical strength, and the ability to withstand extreme pressure differentials. To this end, several membrane frames were singularly epoxy glued to a KF flange, which was then mounted on the inlet of a Helium Leak Detector (HLD). The system was then pumped to about $2.2 \cdot 10^{-10}$ mbar, so that the SiC membrane was the only separation between the inside vacuum environment and atmospheric pressure outside. Helium was sprayed on the surface of the membrane, so that the HLD would reveal membrane porosity or, in the worst case, membrane failure. Table 1 summarizes the results.

As can be seen from the table, the deposition method does not introduce pinholes, and the resulting layer is not stressed. As a reference, a comparison between 100 nm commercial SiN and 100 nm thick SiC membranes, both 1 x1mm² in side, resulted in burst pressure of 1100 mbar and 1400 mbar for commercial SiN and SiC respectively, thus confirming the mechanical robustness of the material.

		Membrane size (side, mm)				
SiC Thickness (nm)		0.3	1	1.5	2	5
	17	OK	NOK			
	50	OK	OK	OK	NOK	
	100	OK	OK	NT	OK	
	150	OK	OK	NT	OK	
	200	OK	OK	NT	OK	NOK

Table 1: summary of porosity and mechanical strength tests of SiC membranes of different thicknesses and different sizes. All the membranes were square. The size in the table refers to the side of the membrane. OK: the membrane passed the test; NOK: the membrane broke upon evacuation; NT: not tested.

Atomic Force Microscopy (AFM) measurements were taken on several 5x5 microns areas of different sample, in contact mode with soft cantilevers (CSC38B Mikromasch, tip radius <10nm, spring constant 0.03 N/m) on a XE-100 instrument (Park Instruments). They have been performed in order to assess the rms roughness of the material, as this can contribute to radiation scattering, as well as constituting an important element for cell adhesion. Figure 1 reports two representative examples of the acquired images. All the images taken, including the ones in Figure 1, show no pinholes, as well as an rms roughness of about 2 nm over the whole image.

Figure 1: AFM images of 2 samples, showing no pinholes. The rms roughness as computed over the images is about 2 nm.

2.3 Cell growth tests

Human Embryonic Kidney (HEK) cells HEK293T have been cultured both over SiC and SiN membranes, in order to test how cells adhere on the two materials. HEK293T cells were grown in DMEM (Thermofisher Scientific, OR, USA) containing 150 U mL⁻¹ penicillin/streptomycin (Sigma–Aldrich) and 10% Fetal bovine serum (Sigma-Aldrich, St. Louis, MO, USA) at 37°C in 5% CO₂. After an incubation of 2 days, the cells have been washed twice with Phosphate Buffer Solution (PBS), then fixed in ParaFormAldehyde (PFA) for 30 seconds, rinsed twice in physiological solution, and

dried under a nitrogen stream. Figure 2 shows visible light images of the cells after 2 days of incubation on the two substrates.

Figure 2: optical images of HEK cells after 2 days of incubation. (a) cells on silicon nitride and (b) cells on silicon carbide.

Figure 2 shows striking differences in the number of cells, their size and shape, on silicon carbide as compared to silicon nitride. HEK293T cells clearly grow and adhere much better on SiC, and they maintain size and shape comparable to the ones detected over standard glass/plastic supports, strongly suggesting a better SiC biocompatibility.

2.4 Infrared and soft X-rays transmission measurements.

The infrared (IR) and X-ray transmissions have been respectively measured at the SSSI [8] and TwinMic [9] beamlines of Elettra synchrotron light source in Basovizza, (TS), Italy. The IR measurements were acquired in the $4000\text{-}900\text{ cm}^{-1}$ region, while the X-ray transmission was measured at 780 eV incoming photon energy only).

IR transmission measurements are reported in Figure 3; the results indicate comparable transmission for both SiC and SiN membranes. In particular, a comparison between 100 nm-thick SiC and SiN membranes is reported in the inset, showing a slightly higher transmission in the case of the SiC membrane for wavenumbers higher than 2300 cm^{-1} .

Figure 4 reports the transmission of SiC and Si_3N_4 layers as a function of photon energy, between 700 and 860 eV. The data has been extracted from the Center for X-Ray Optics (CXRO) online tool [10] using standard values for the density.

The transmission was measured at the single photon energy of 780 eV. This energy was selected because of its usage at TwinMic beamline for high resolution imaging. Both the membranes were nominally 100 nm thick. The measurements have been made acquiring 100 points at different membrane locations, and then averaging

them out. The transmission for SiC at 780 eV is $91 \pm 2\%$, while the transmission for SiN is $90 \pm 2\%$. The discrepancy between calculated and measured SiN transmission depends strictly on the stoichiometry of the material. As pointed out in literature [11], the specific stoichiometry depends on the deposition conditions. The typical low stress silicon nitride used to make membranes is actually closer to SiN_x , i.e. a silicon-rich silicon nitride, rather than Si_3N_4 , and the different stoichiometries accounts for the discrepancy between calculated and measured transmission.

Figure 3: Infrared transmission over the 900-4000 cm^{-1} range for a variety of SiC thicknesses. The red curve is the reference for 100 nm thick SiN. In the inset, a comparison between 100nm SiC and SiN (always in red) is shown for the 900-2000 cm^{-1} spectral region.

Figure 4: calculated X-ray transmission for SiC (blue line) and SiN (green line). The numerical values are the theoretical transmissions at 780 eV.

IR chemical mapping and Scanning Transmission X-ray Microscopy (STXM) images of the cultured cells were taken at the SSSI and TwinMic beamlines respectively, and are reported in Figure 5 and 6. The IR chemical mapping allowed comparing directly the presence in the cells of typical chemical moieties, while the STXM images assesses the morphology of single cells with submicron resolution.

The optical images of HEK cells grown on SiN and SiC membranes are reported in Figure 5A and 5C respectively. The chemical images in Figure 5 (B and D) have been obtained by integrating the vibrational band in the $1150\text{-}985\text{ cm}^{-1}$ spectral range. This range is relevant for sugar and sugar-phosphate moieties vibrations, typical of carbohydrates and nucleic acids [5,6].

Figure 6 shows the STXM images of two different areas on a 100 nm thick SiC membrane. The left hand side one shows a single cell, while the right hand side depicts a group of cells. Both the images have been acquired at 780 eV incoming photon energy, the pixel size is 300 nm and the dwell time was 80 ms.

Figure 5: Optical and chemical mapping of HEK293T cells on SiN (A, B) and on SiC (C, D) membranes. A: optical image of HEK293T cells grown on SiN membranes and B: related chemical image. Scale bar from 0 (Min) to 4.1 a.u. (Max). C: optical image of HEK293T cells grown on SiC membranes and C: related chemical image. Scale bar from 0 (Min) to 2.5 a.u. Band integral in the $1150\text{-}985\text{ cm}^{-1}$ range

3. Discussion

The quality of epitaxially grown silicon nitride membranes has been assessed in several different ways, at several different thicknesses, with different membrane size and compared against the more widely used silicon nitride. From a fabrication standpoint, SiC is as easy as SiN to handle, and requires minimal adjustments to standard SiN membranes fabrication protocols. Mechanically, SiC exhibits a higher tolerance to pressure than SiN, with a typical 30% increase in robustness, given the same nominal thickness and the same window size. The tests performed with a Helium Leak Detector allowed establishing the absence of pinholes in the deposited films, as the membranes were able to separate the HLD vacuum from atmospheric pressure. The AFM scans confirmed the absence of pinholes on all the imaged samples, and allowed to measure a relatively low rms roughness (around 2 nm).

Figure 6: STXM images of HEK cells grown on SiC. Both images were acquired at 780 eV photon energy. The pixel size in each is 300 nm, with a dwell time of 80 ms.

The cell growth tests demonstrate quite clearly a substantial improvement in cell adhesion to the substrate as compared with SiN. As can be seen from Figure 2, the number of cells and their shape on the SiC membrane is far larger than on the SiN one. This is also suggested by the large triangular shape of the cells on SiC, while on SiN they are round, and quite isolated, hinting at some kind of difficulty in cellular replication on SiN. Since the culture conditions are exactly the same, and this happened in all the substrates, the indication is that SiC is more cell-friendly than SiN.

The IR maps, shown in Figure 5, display a direct comparison between the spectral responses related to carbohydrates and phosphate moieties in the imaged area [12,13]. The selected spectral region can be used for determining the spatial extent of the cell nuclei. The chemical image shows higher intensity (red-pink areas) at nuclear level. Both optical and chemical images shown that cells grown on SiC are bigger in extent and firmly adherent onto it, letting to postulate an overall better biocompatibility of SiC as compared to SiN. As a consequence of the better adhesion, the cells are

flatter, and the IR signal intensity at the nuclear region for cells grown on SiC is lower (2.5 a.u.) than that on SiN (4.1 a.u.). Indeed, cells grown onto the latter membranes are round shaped, and the higher intensity of IR signal suggests that they are thicker as a consequence of a worst adhesion onto the membrane. Despite the much shorter optical path on adherent HEK293T, the spectral region below 1200 cm^{-1} was easily accessible, due to the superior transparency of SiC in this spectral region, as can be appreciated in Figure 3: the transparency of SiC extends beyond 1200 cm^{-1} , up to $\sim 1000\text{ cm}^{-1}$, while the one of SiN quickly diminishes in this region due to Si-N stretching mode absorption.

The higher transmission at X-ray and IR wavelengths, as compared to SiN of the same nominal thickness, makes SiC a good substrate for transmission IR measurements, as well as X-ray microscopy, X-ray fluorescence and potentially also CDI measurements. Unrelated to this work, the same membranes have been successfully tested at Free Electron Lasers [14].

4. Conclusions

The present work demonstrates the usability of SiC as a substrate for synchrotron X-ray and IR measurements. The combination of higher transmission and higher mechanical strength than SiN implies that one can choose a SiC membrane with the same SiN transmission, but much higher mechanical strength, as in order to exhibit the same transmission, the SiC layer would be significantly thicker than the SiN one. As an alternative, one could choose the same mechanical stability, but with a much improved transmission.

Further, the biocompatibility suggested by the measurements indicates that SiC is a superior choice for bio-oriented studies.

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References

- [1] C. Visser, J. E. Uglow, D. W. Burns, G. W. Microelectronic, 1987, "A new silicon nitride mask technology for synchrotron radiation X-ray lithography: First results," *Elsevier*, vol. 6, no. 1, pp. 299–304, Dec. 1987.
- [2] <http://www.norcada.com/products/xray-microscopy/>
- [3] J. Kirz, C. Jacobsen, and M. Howells, "Soft x-ray microscopes and their biological applications," *Quarterly Reviews of Biophysics*, vol. 28, pp. 33–33, 1995.
- [4] E. A. Carter, B. S. Rayner, A. I. McLeod, L. E. Wu, C. P. Marshall, A. Levina, J. B. Aitken, P. K. Witting, B. Lai, Z. Cai, S. Vogt, Y.-C. Lee, C.-I. Chen, M. J. Tobin, H.

- H. Harris, and P. A. Lay, "Silicon nitride as a versatile growth substrate for microspectroscopic imaging and mapping of individual cells," *Molecular BioSystems*, vol. 6, no. 7, pp. 1316–1322, Jun. 2010.
- [5] M. Altissimo, L. Pascolo, R. Delfino, and M. Salome, "Tracing exogenous Gd and its effects in single Chang cells," *Nuclear Instruments and ...*, 2009.
- [6] M. M. Seibert, S. Boutet, M. Svenda, T. Ekeberg, F. R. N. C. Maia, M. J. Bogan, N. Timneanu, A. Barty, S. Hau-Riege, C. Caleman, M. Frank, H. Benner, J. Y. Lee, S. Marchesini, J. W. Shaevitz, D. A. Fletcher, S. Bajt, I. Andersson, H. N. Chapman, and J. Hajdu, "Femtosecond diffractive imaging of biological cells," *J. Phys. B: At. Mol. Opt. Phys.*, vol. 43, no. 19, p. 194015, Oct. 2010.
- [7] Wang, L.; Dimitrijević, S.; Jisheng Han; Iacopi, A.; Hold, L.; Tanner, P.; Harrison, H.B., *Growth of 3C-SiC on 150-mm Si(100) substrates by alternating supply epitaxy at 1000deg C*, Thin Solid Films, Vol. 519, No. 19, 2011, 6443-6
- [8] S. Lupi, A. Nucara, A. Perucchi, P. Calvani, M. O. J. B, 2007, "Performance of SSSI, the infrared beamline of the ELETTRA storage ring," *osapublishing.org*.
- [9] A. Gianoncelli, G. Kourousias, L. Merolle, M. Altissimo, and A. Bianco, "Current status of the TwinMic beamline at Elettra: a soft X-ray transmission and emission microscopy station.," *J Synchrotron Radiat*, vol. 23, no. 6, pp. 1526–1537, Nov. 2016.
- [10] <http://www.cxro.lbl.gov>
- [11] P. Temple-Boyer, C. Rossi, E. Saint-Etienne, and E. Scheid, "Residual stress in low pressure chemical vapor deposition SiNx films deposited from silane and ammonia," *Journal of Vacuum Science & Technology A: Vacuum, Surfaces, and Films*, vol. 16, no. 4, pp. 2003–2007, Jul. 1998.
- [12] Banyay M, Sarkar M, Gräslund A. *A Library of IR Bands of Nucleic Acids in Solution*, July 2003 Biophysical Chemistry 104(2):477-88 DOI10.1016/S0301-4622(03)00035-8
- [13] P Zucchiniati, E Mitri, S Kenig, F Billè, G Kourousias, DE Bedolla, L Vaccari. *Analytical Chemistry* 88 (24), 12090-12098, 2, 2016.
- [14] Mincigrucci et al, manuscript in preparation.