



# BioMEMS Materials and Fabrication Technology:

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## *Nanoporous SiC: A Candidate Semi-Permeable Material for Biomedical Applications\**

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**Abstract.** We have fabricated free-standing SiC nanoporous membranes in both *p*-type and *n*-type material. We showed that these membranes will permit the diffusion of proteins up to 29000 Daltons, while excluding larger proteins. By using radioactively labeled albumin, we also show that porous SiC has very low protein adsorption, comparable to the best commercially available polymer nanoporous membrane.

**Key Words.** silicon carbide, ultrafiltration, membrane, microdialysis, biosensor

### Introduction

The growing capability of microsystems to incorporate sophisticated electronics with mechanical parts and microfluidics is enabling multiple new applications for microdevices. Microsystem interfaces with liquid environments allow environmental monitoring, chemical and biological process monitoring, and various medical applications. Considerable effort has been devoted to developing porous silicon membranes permeable to liquids that can act as such an interface. Microfabricated porous silicon membranes (Chu et al., 1999) have been applied to an implantable artificial pancreas (Desai et al., 1998) and kidney (Fissell et al., 2003) and oral drug delivery systems (Tao and Desai, 2003). In the artificial pancreas, the membrane serves to contain transplanted insulin producing cells, allowing the release of insulin but protecting the cells from attack by immunoglobulin. In the kidney application, the membrane acts as a support for kidney cells and as a blood filter that retains serum proteins while allowing smaller waste substances out. In drug delivery, the membrane allows the

targeted diffusion of a drug within the bowel by release of the drug in close proximity to the wall of the bowel.

Both Si and SiC appear to be acceptably biocompatible materials for tissue implantation. Kotzar et al. (2002) evaluated materials used in microelectromechanical systems (MEMS) devices for biocompatibility. These included single crystal silicon, polysilicon (coating, chemical vapor deposition, CVD), single crystal cubic SiC (3C-SiC or  $\beta$ -SiC, CVD), and titanium (physical vapor deposition). They tested for extractable material using both aqueous and non-aqueous solvents, performed cytotoxicity and tissue implantation studies. They found very low levels of extractable material, very low cell cytotoxicity and no significant tissue reactions with all four materials. They concluded that the tested Si, SiC and titanium were biocompatible and equivalent, in these experiments. Other studies have also confirmed the good tissue biocompatibility of both silicon, usually tested as devices or wafer chips (Jahnsen et al., 1999; Leoni et al., 2002; Schmidt et al., 1993) and SiC, usually tested as a coating made by CVD (Bolz and Schalldach, 1990; Naji and Harmand, 1991; Santavirta et al., 1998).

SiC coatings may have an advantage over silicon in having less protein adhesion and less platelet aggregation when exposed to blood. These properties may translate into less protein biofouling and better compatibility for

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intravascular applications. Auditore et al. (2002) created surfaces coated with amorphous mixtures of hydrogenated silicon and carbon. They used plasma enhanced chemical vapor deposition (PECVD) to vary the ratio of silicon and carbon in the material. They reported that silicon-rich surfaces adsorbed more albumin, and that this tendency decreased with increasing carbon concentration. Weisenberg and Mooradian (2002) showed that silicon and silicon nitride had significantly higher levels of platelet adhesion than silicon dioxide, paralone or SU-8 polymers. They did not directly compare silicon to SiC. To decrease the protein binding and platelet adhesion of silicon surfaces, polyethylene glycol has been grafted to silicon (Sharma et al., 2002; Zhang et al., 2001). SiC has relatively low levels of fibrinogen and fibrin deposition when contacting blood (Takami et al., 1998). These proteins promote local clot formation; thus, the tendency not to adsorb them will resist blood clotting. It is now well established that SiC coatings are resistant to platelet adhesion and clotting both *in vitro* and *in vivo*. Bolz and Schaldach (1990) evaluated PECVD amorphous SiC for use on prosthetic heart valves. They showed decreased thrombogenicity of an amorphous layer of SiC compared to titanium. Several other studies showed that hydrogen-rich amorphous SiC coating on coronary artery stents is anti-thrombogenic (Bolz et al., 1996; Bolz and Schaldach, 1990; Carrie et al., 2001; Monnick et al., 1999). In clinical studies with SiC coated stents, one randomized study (497 patients) showed no benefit (Unverdorben et al., 2003). Three other studies (total of 2,125 patients), showed a benefit that was attributed to the SiC-coated stent (Elbaz et al., 2002; Hamm et al., 2003; Kalnins et al., 2002). In a direct comparison of silicon wafers and SiC-coated (PECVD) silicon wafers for blood compatibility, both appeared to provoke clot formation to a greater extent than diamond-like coated silicon wafers; silicon was worse than SiC-coated silicon (Nurdin et al., 2003). It is not known if the lower protein adsorption and clot resistance of PECVD deposited SiC will translate to porous SiC, the subject of the present study.

In this work, we introduce porous SiC as a potential candidate for *in vivo* membrane applications. We focus on the suitability of porous SiC with pore sizes of tens of nanometers as a membrane material for implantable microdevices for biomedical applications.

In evaluating nanoporous SiC, we measured two important properties: first, the ability to allow the diffusion of relatively small proteins while preventing the diffusion of larger proteins, and second, resistance to the adsorption of protein to the surface of the material, which will contribute to fouling of the membrane. Fouling of membranes is a major problem with implanted biosensors (Zhang et al., 2000).

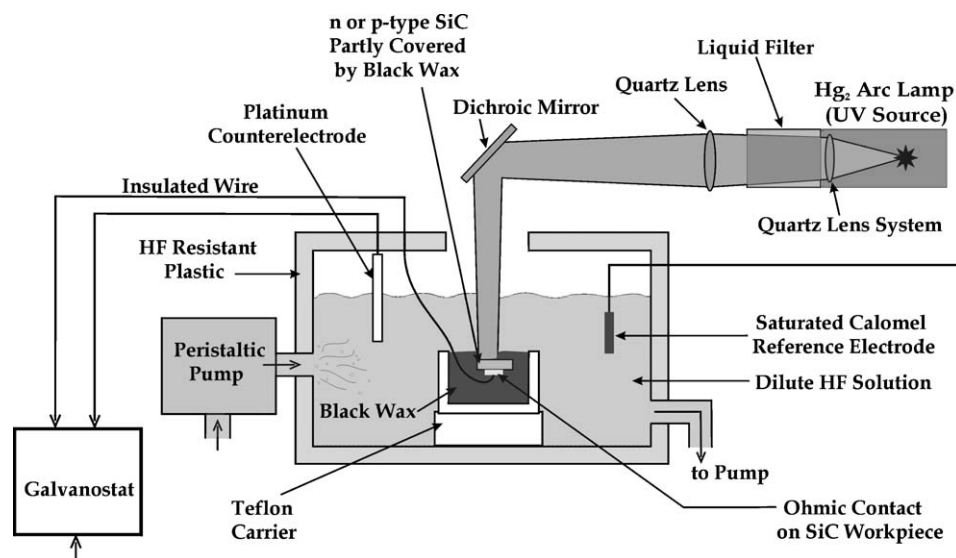
## Methods

The SiC samples used in this study are *n*-type and *p*-type 300–400  $\mu\text{m}$  thick 6H SiC crystals. Two-inch diameter wafers were obtained from Cree, Inc. (www.cree.com) and cut into  $1 \times 1$  cm square pieces prior to processing. In general, nominal doping for *n*-type crystals ranges from  $3 \cdot 10^{18}$  to  $1 \cdot 10^{19} \text{ cm}^{-3}$  while *p*-type samples are doped at about  $2 \cdot 10^{18} \text{ cm}^{-3}$ .

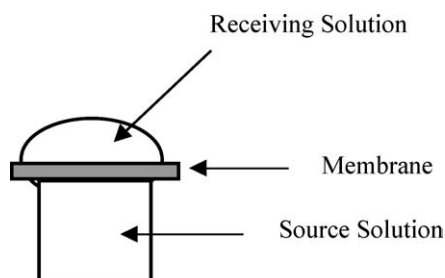
The porous structures are obtained by electrochemical etching, described for SiC by Shor and Kurtz (1994). We operated in the anodic regime using a standard three-electrode arrangement (electrodes are about 5 cm apart) where a SiC crystal serves as the working electrode, a saturated calomel electrode as the reference, and a platinum disk as the counter electrode. The experimental setup is shown in Figure 1. The etching is performed in a 5% (final concentration) aqueous hydrofluoric (HF) electrolyte mixed with 5% ethanol where the chemicals are measured by weight. All the *p*-type samples are anodized in the dark, whereas all the etching of the *n*-type samples is done with UV light assistance provided by a 350 W mercury arc lamp. More information about the etching conditions and the corresponding porous structures can be found elsewhere (Bai et al., 2003). The precise reason for the different morphologies produced by the different anodization conditions is under investigation.

After the porous SiC structure has been prepared, we separate the film from the remaining substrate by applying high current density (up to  $100 \text{ mA/cm}^2$ ). This type of technique for separating porous SiC membranes from the original substrates was described for SiC by Kim et al. (2000). We are able to realize free-standing porous SiC membranes of 20 to 110 micron thickness of both *n*- and *p*-types.

For measuring protein permeability, free-standing nanoporous SiC membranes were glued onto circular polystyrene plastic supports and placed into a small fluidic chamber. The schematic of the experimental arrangement is shown in Figure 2. The glue was made by mixing approximately 1 part polystyrene and 4 parts methylene chloride by volume. This glue was able to withstand months of exposure to water without softening and it was not permeable to proteins, as demonstrated by many earlier membranes that were tested by us with no protein permeation using this glue. The plastic supports exposed approximately  $3 \text{ mm}^2$  of the membrane surface.  $350 \mu\text{L}$  of protein-containing solution was placed under the membrane in contact with it. This was the source solution. Air was excluded from the membranes by soaking them in ethanol, and then water (double distilled in glass) prior to use. Bubbles were excluded from the chambers by carefully placing the membrane onto the surface of the



**Fig. 1.** The schematic of the experimental setup for the electrochemical etching. The sample with an attached electrode is encapsulated into black wax and placed into an electrolyte bath. The potential and current applied to the sample are controlled by a galvanostat. UV illumination is used to anodize *n*-type SiC.



**Fig. 2.** The schematic of the experimental setup for testing protein diffusion through porous SiC membranes. The source solution contains the proteins to be tested. These proteins diffuse through the membrane into the receiving solution, which is sampled periodically.

liquid in the test chamber. Since the membranes are thin and translucent, the presence of even very tiny bubbles could be observed through the membranes with a dissecting microscope. The mix was stirred continuously with a magnetic stir bar. Buffer (30  $\mu\text{L}$  of phosphate buffered saline, pH 7.4, 0.1% sodium azide) was placed on top of the membrane, covering its exposed surface. This was the receiving solution. Molecules within the source solution below the membrane diffused through the membrane into the receiving solution above. Samples of the receiving solution were removed (7  $\mu\text{L}$ ) at 0, 2, 4, 6, and 18 hours. After each sample was taken, volume was replaced with 7  $\mu\text{L}$  of fresh buffer, with mixing. This method of sampling introduces an error, because a significant amount of protein (23%) is removed for sampling at each time point. The data therefore *underestimate* the actual amount of protein recovery through the membrane. This method

was chosen to minimize confounding effects caused by protein biofouling and to avoid risking breakage of the membranes. By removing samples serially at each time point we were able to avoid depleting the protein reservoir below the membrane significantly and to get multiple data points upon the first exposure to protein. Given the high concentration of proteins, using a larger reservoir to screen many membranes would have been too costly. Having to remove the membrane would risk drying the membrane with protein on it. Biofouling is a significant problem with any nanoporous membrane exposed to high protein concentrations. Commercially available membranes that contact protein are generally not reused. Furthermore, when working with commercial polymer membranes, we have observed significantly reduced protein throughput starting in less than 3 hours (unpublished data). Also, at this stage, we have no method worked out to safely clean the SiC membranes of protein without possibly damaging them, denaturing the proteins within the pores, precipitating detergent or other solvent within the pores, or damaging the glue bond. Also, cleaning is likely to be incomplete, given the tortuosity of the channels, and the large surface area exposed to protein. Moreover, manipulation of these very thin membranes risks breaking them, as we have seen a number of times. Given these complications, we chose this simple method of sampling for a preliminary estimation of protein permeability through SiC membranes, realizing that the permeability shown here is an underestimate.

The chamber was sealed with Parafilm™ in order to prevent evaporation. Protein concentrations in the

receiving solution were measured by sodium dodecyl sulfate capillary electrophoresis (PACE/MDQ system, Beckman-Coulter, San Jose, California).

The results are expressed as the percentage of relative recovery. The relative recovery is defined as the ratio of the concentration of a protein in the receiving solution to the concentration in the source solution. A relative recovery of 100% for a protein would indicate that the protein had achieved the same concentration in the receiving solution as in the source solution. Depletion of protein from the source solution was negligible. The receiving solution was 8.6% of the volume of the source solution (30/350  $\mu$ l) and the receiving solution only attained 20% of the concentration of the source solution with the most permeable protein (myoglobin). Thus the depletion of the source solution was roughly 1.7% (8.6%/5) for myoglobin. Other proteins were depleted less.

For an investigation of the capability of larger molecules to diffuse through the membranes, we used six test proteins ranging in molecular weight from 17000 to 80000 Dalton (Da). These were mixed together in the test solution. All proteins were obtained from Sigma-Aldrich (St. Louis, Mo.) The proteins, their catalog numbers, molecular weights and final concentrations are: myoglobin (MYO, #70025 17000 Da, 60 micromolar), soybean trypsin inhibitor (STI, #9767, 20000 Da, 60 micromolar), carbonic anhydrase (CAR, #C2273, 29000 Da, 30 micromolar), ovalbumin (OVA, #A5503, 45000 Da, 30 micromolar), bovine serum albumin (ALB, #A7517, 66000 Da, 30 micromolar), and human transferrin (TFN, #T8158, 80000 Da, 30 micromolar).

We compared the non-specific adsorption of protein to nanoporous SiC and to a commercially available polymer membrane specifically designed to resist protein deposition. ALB was used in these tests. ALB is well known to be a "sticky" protein and is commonly used to coat non-biologic materials in order to passivate them. Also, a low protein absorbing commercially available membrane (Omega Membrane, Pall Corporation, #OM100025, 100 kDa molecular weight cut-off) was tested with ALB by others (Strumeyer, D.H.). This allowed a valid comparison of porous SiC to well characterized membranes. The commercial Omega membranes are composed of polyethersulfone (PES), which is also used in many microdialysis catheters (CMA Microdialysis). In comparison, the protein adsorption of conventional, unmodified PES is 24 times greater than the Omega membrane. Also, the accepted previous standard for low protein adsorption was regenerated cellulose. This material has a protein adsorption- three times greater than that of the Omega membrane (Strumeyer, D.H.). In order to compare SiC membranes to the Pall membranes for protein adsorption, the total surface area of each membrane was measured

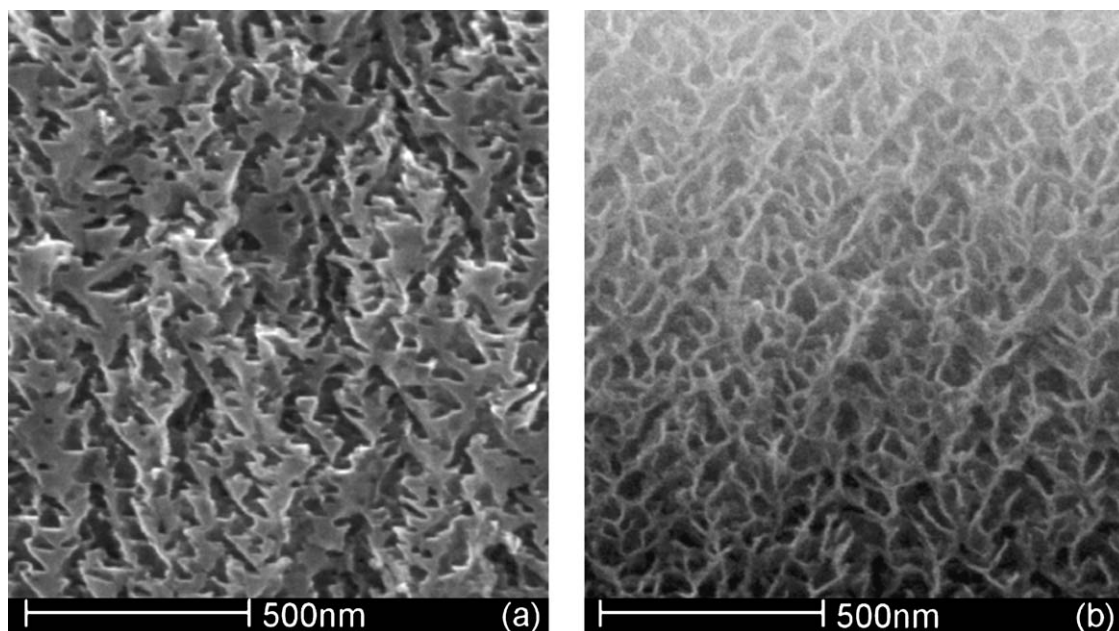
using the National Institutes of Health (NIH) ImageJ software. The front, back and edge areas were added together to get the total surface area. Membranes were then placed in a solution containing unlabeled ALB with tracer  $I^{125}$ -labeled ALB. The concentration of unlabeled ALB was either 15 micromolar (1 mg/mL, one experiment) or 150 micromolar (10 mg/mL, two experiments).  $I^{125}$ -labeled ALB adhering to the membrane was measured using a Packard Cobra-II Autogamma gamma counter. Background was estimated from tubes exposed to tracer  $I^{125}$ -labeled albumin but lacking membranes. Results were normalized for surface area.

## Results

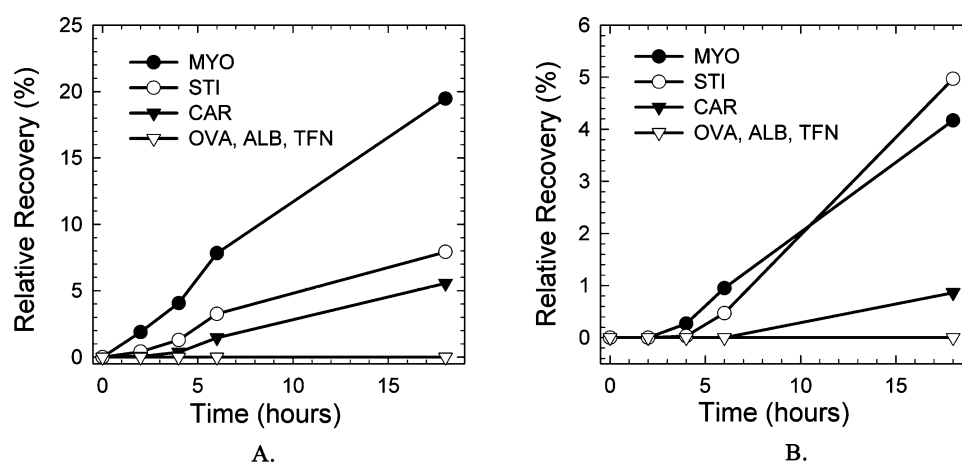
For the current experiments, the two porous SiC morphologies shown in Figure 3 are used. The cross-sectional SEM images were taken in the middle part of the material. Figure 3(a) shows the structure of the tested porous 35  $\mu$ m thick membrane made from *n*-type 6H SiC. Figure 3(b) shows the structure of the tested porous 36  $\mu$ m thick membrane made from *p*-type 6H SiC.

Figures 4(a) and (b) show the results of the diffusion of the six proteins through our *n*-type and *p*-type porous SiC membranes, respectively. Despite considerably different pore structure (see Figure 3), membranes of both *n*-type and *p*-type SiC exclude proteins in the same size range. The *n*-type material allowed much more protein to diffuse through, by a factor of as much as four times (for myoglobin). We have not yet investigated the effect of membrane thickness. Each membrane type passed proteins of up to 29000 Da molecular weight but excluded larger proteins with a molecular weight of 45000 Da and higher. These molecular weights correspond to molecular diameters of less than 4.7 nm (permeable) and greater than 5.0 nm (excluded). The discrimination between the 29 kD and 44 kD proteins is likely complex, and not simply based on size (4.7 and 5.0 nm, respectively). For example, charge, hydrophilic or hydrophobic interactions, as well as dimerization in solution or differential adsorption to the SiC, to the glue, to the plastic tube or membrane backing, etc., could all play roles. This same behavior was observed when we tested the same 6-protein mix with commercial membranes (data not shown), i.e., ovalbumin is excluded to a much greater degree than carbonic anhydrase.

Figure 5 shows the comparative binding of radioactively labeled albumin to porous 6H SiC (*n*-type and *p*-type) versus the Omega membrane from Pall Corporation, adjusted for the area of exposed membrane that contacted the albumin-containing solution. The porous 6 H SiC membranes bound a similar amount of ALB as the best available commercial polymeric membrane per area exposed.



**Fig. 3.** The cross-sectional SEM images taken from pieces of porous membranes used for the tests: (a) n-type 6 H SiC and (b) p-type 6 H SiC. The p-type porous structure has a more feathery appearance, corresponding to more material removal. These membranes are more fragile than the n-type membranes.



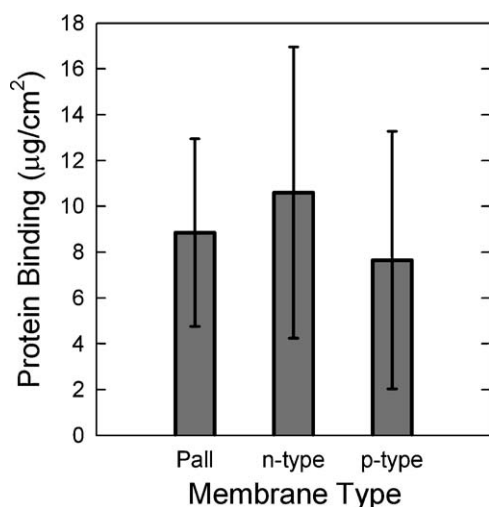
**Fig. 4.** (A) Relative recovery of different proteins expressed as a percentage of the original concentration after passing through the n-type porous 6 H SiC membrane shown in Figure 3(a). (B) Relative recovery of different proteins expressed as a percentage of the original concentration after passing through the p-type porous 6H SiC membrane shown in Figure 3(b).

## Discussion

The observations in this study were purely empirical. At this early phase of development of porous SiC membranes, we have not modeled the membrane nor diffusion through it. Also, at this stage, we do not have a method to quantify the porosity of the SiC.

It has been shown (Bai et al., 2003), that nanoporous SiC membranes can be made with a wide variety of pore

sizes, shapes and densities by varying the conditions of pore formation. Pore diameters can range from tens of nanometers up to two microns. The material tested in this study passed proteins of up to 29000 Da through the membrane while those of 45000 Da and higher were excluded. The size range of proteins able to cross the membranes includes many important cell signaling molecules, which tend to be small. This suggests that the material is may be suitable for biosensor and other applications where



**Fig. 5.** Comparison of albumin  $I^{125}$ -labeled protein binding to porous *n*- and *p*-type SiC membranes versus the best commercially available low-adsorption polymer membrane. Error bars represent the standard deviation of 3 experiments using different samples of conventional, *p*-type or *n*-type membranes.

permeability of small molecules and small proteins and exclusion of larger molecules is desirable. The amount of protein passed through these membranes was relatively small. It is not understood why the *p*-type material, which appears to be more porous than the *n*-type material had significantly less permeability to proteins. However, we expect that the ability to vary many parameters in the pore formation process of these membranes will allow us to increase protein diffusion. The size range of proteins that cannot diffuse through the membranes enables protection of transplanted cells from immunoglobulin (immunoisolation). Also, as a support material for artificial kidneys, these membranes will retain valuable blood proteins such as albumin and globulins, while allowing smaller (waste) molecules to pass. Nanoporous SiC has a low protein adsorption comparable to the best commercially available polymeric membranes specifically designed for low protein adsorption. This resistance to protein fouling is another desirable property of this material. Furthermore, SiC has previously been shown to have excellent biocompatibility and hemocompatibility. The combination of these important properties of nanoporous SiC suggests that this material could be very useful in medical microsystem applications.

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