

Porous Silicon Carbide as a Membrane for Implantable Biosensors

A.J. Rosenbloom^{1*}, Y. Shishkin², D.M. Sipe¹, Y. Ke², R.P. Devaty²,
and W.J. Choyke²

¹ Molecular Biosensors & Imaging Center, Carnegie Mellon University, Pittsburgh, PA 15213, USA

² Department of Physics and Astronomy, University of Pittsburgh, Pittsburgh, PA 15260, USA

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Abstract. This work demonstrates the creation of free-standing, nanoporous SiC membranes, and their ability to pass molecules of biologic interest. We have shown that proteins up to 29000 Daltons in molecular weight can pass through porous SiC. Also, we have shown that porous SiC resists protein adsorption comparable to the best commercially available polymer membrane that has been specifically developed to avoid protein adsorption.

Introduction

An emerging area of biotechnology is the development of implantable sensors. These sensors will measure molecules within the water that surrounds the cells comprising tissues and organs. The aim is to recognize disease processes in their early stages. New materials are needed in order to create an interface between microdevices and living tissues. In this work, we investigate the suitability of porous SiC for such an interface.

A simple technique, known as microdialysis, has demonstrated the usefulness and basic principles of sampling molecules in tissue fluids by diffusion in living animals and man. This technique allows sampling of the microenvironment between cells, which is known as the interstitial or intercellular fluid. In microdialysis, a buffer solution is pumped through a small semi-permeable tube that has been placed into living tissue. Molecules in the interstitial fluid diffuse into the buffer and are pumped out and collected for measurement (Figure 1). Recently, the recovery of larger than 1000 Dalton molecules (one Dalton (Da) equals one hydrogen atomic mass), specifically proteins, by microdialysis has been demonstrated [1]. The ability to use microdialysis to obtain interstitial proteins vastly expands the pool of possible molecular targets. However, in practice, microdialysis as currently done allows only short term monitoring. The longest reported use so far has been for 3 weeks [2]. In order to achieve long-term monitoring of tissues and organs, permanent or semi-permanent implantable sensors will be required.

The structure of nearly all implantable biosensors includes a covering membrane. This membrane contains and protects the sensor elements and serves as an interface with the tissue. It will be necessary for indwelling biosensor membranes to be biocompatible, i.e., able to integrate with the tissue environment without evoking inflammation, scar formation, clot formation in blood vessels, or other undesirable tissue reactions. Another desirable membrane property is control over porosity. Uniform, well-controlled pores in the material will allow small proteins and metabolites to pass, while excluding cells and larger molecules such as immunoglobulins, which

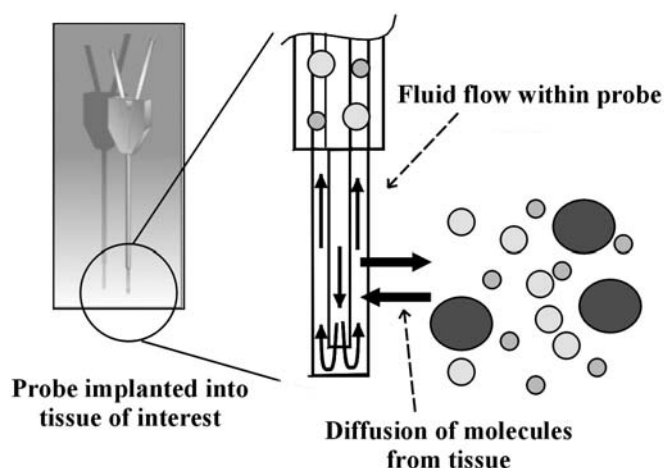


Figure 1. Principles of microdialysis.

* corresponding author: rosenbloomaj@ccm.upmc.edu

could damage the sensor. High porosity is important for high throughput of molecules. This allows efficient sensing of species present in low quantities. Equally important is the ability to resist excessive protein deposition on the material of the membrane. Protein deposition can lead to clogging of the pores and a decrease or cessation of the flow of molecules to the sensor (biofouling). These technical problems remain a major barrier to the fabrication and deployment of implantable sensors [3]. Another desirable quality for sensor membranes is chemical resistance, since the tissue environment can be remarkably corrosive. Finally, it is important that biosensor membranes can interface with silicon microchips to take advantage of the growing capability of these devices in microfluidics, sensing, data processing and data transmission. Nanoporous SiC possesses many desirable properties for this application. It has proven biocompatibility, as evidenced by successful use as a coating on vascular stents [4] that are inserted into coronary arteries after balloon angioplasty to maintain arterial patency. SiC is very chemically resistant and can be integrated with microchips. This work demonstrates that porous SiC is permeable to small proteins but that it excludes larger proteins. Also, the protein adsorption of these SiC membranes was measured and found to be comparable to the best commercially available polymeric ultrafiltration membranes designed for use with proteins. These membranes have been specifically developed for low protein binding.

Experimental Results and Discussion

The preparation of porous SiC layers is done by electrochemical etching of SiC crystals in aqueous HF electrolyte mixed with ethanol. The etching is conducted in the anodic regime using a standard three-electrode cell configuration with SiC serving as the working electrode. A saturated calomel electrode is used as a reference and a platinum disk as a counter electrode. The p-type porous SiC has the algae-like branched morphology [5,6] which is obtained in the dark under 5 mA/cm^2 current density. The porosity is about 55% and is uniform through the layer. The porous chevron/dendritic type of pore morphology is obtained for the n-type porous SiC [5], which requires illumination of the front surface with UV light and current densities up to 15 mA/cm^2 . The porous n-type SiC structures have a porosity gradient (about 40% at the top and 15% at the bottom). After the porous structures have been prepared, we separate them from the original substrates. The membrane separation is done by the high-current separation technique, which allows us to realize free-standing porous membranes of 20 to 120 microns thickness of both n- and p-types. The prepared membranes are studied in cross-section using a Philips XL 30 FEG scanning electron microscope.

The free-standing nanoporous films are glued onto circular polystyrene plastic supports that exposed approximately 3.1 mm^2 of the membrane surface. The membranes were placed into a small fluidic chamber. Solutions to be tested (350 μL volume) were placed under the membrane in contact with it. Bubbles were excluded and the mix was stirred continuously with a magnetic stir bar. Buffer (30 μL phosphate buffered saline, pH 7.4, 0.1% sodium azide) was placed on top of the membrane, covering the exposed area. Molecules within the test solution diffused through the membrane into the buffer above. Samples (7 μL volume) of the top buffer were removed at 0, 2, 4, 6 and 18 hours. After each sample was taken, volume was replaced with 7 μL of fresh buffer, with mixing. Samples were analyzed for protein content using capillary electrophoresis. The fluidic chamber was sealed with ParafilmTM in order to prevent evaporation.

Preliminary experiments indicated that n-type porous SiC was permeable to NaCl, glucose, and Bovine Serum Albumin (results not shown). A more in-depth investigation of the capability of larger molecules to diffuse through the membrane was done using six proteins (Figures 2 and 3). The test proteins ranged in molecular weight from 17000 to 80000 Dalton (Da). The proteins were: myoglobin (MYO, 17000 Da), soybean trypsin inhibitor (STI, 20000 Da), carbonic anhydrase (CAR, 29000 Da), ovalbumin (OVA, 45000 Da), bovine serum albumin (ALB, 66000 Da), and human transferrin (TFN, 80000 Da). Protein concentrations were measured by capillary electrophoresis

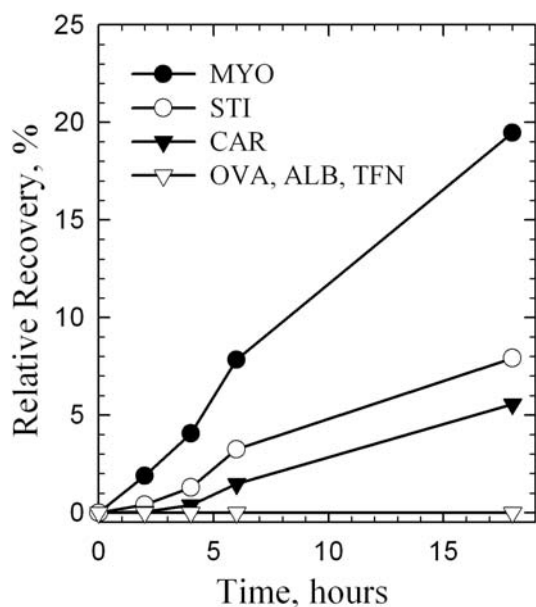


Figure 2. Recovery of proteins through n-type nanoporous SiC.

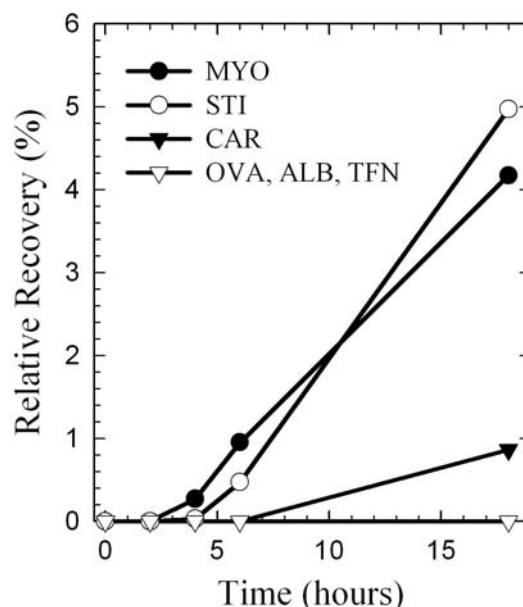


Figure 3. Recovery of proteins through p-type nanoporous SiC.

(Beckman-Coulter PACE/MDQ system, (Fullerton, CA)) in sodium dodecyl sulfate (which separates proteins based on molecular weight).

The results are expressed as the percent relative recovery. Relative recovery (%) is obtained by dividing the concentration of a protein recovered in the buffer on top of the membrane by its concentration in the protein solution on the bottom of the membrane, and multiplying by 100. A relative recovery of 100% for a protein would indicate that the protein had achieved the same concentration in the collection buffer as in the original protein solution. Two types of membranes were examined: n-type SiC and p-type SiC.

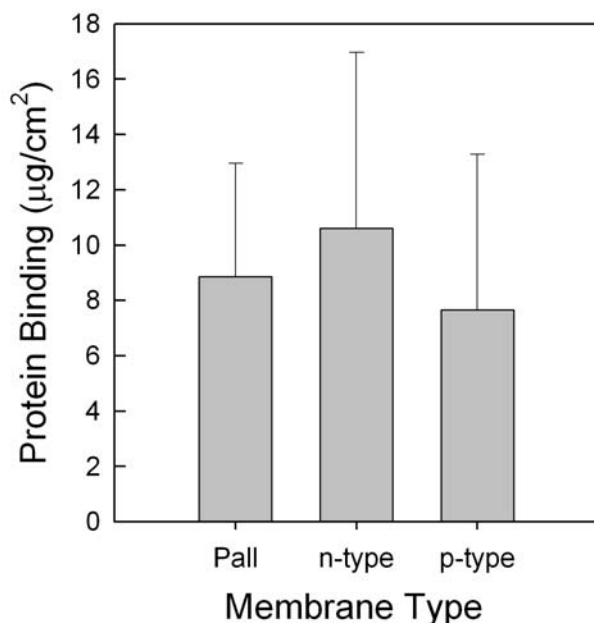


Figure 4. Comparison of albumin binding to porous SiC films versus best commercially available low-adsorption polymer membrane.

Despite considerably different pore structures, membranes of both p-type and n-type excluded proteins in the same size range. 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 ≤ 4.7 nm (permeable) and ≥ 5.0 nm (excluded). Although individual pores appeared considerably larger than the diameters of the proteins excluded, the tortuosity of the passages through the material likely accounts for the inability of the larger proteins to negotiate a way through the SiC membranes. The n-type material allowed much more protein to diffuse through, by a factor of as much as four times (for Myoglobin) greater than the p-type. We have not yet investigated the effect of membrane thickness.

Bovine serum albumin (BSA) was used to estimate protein adsorption onto nanoporous SiC. BSA is well known to be a “sticky” protein and is commonly used to coat non-biologic materials in order to passivate them. Commercially available membranes designed to resist protein adsorption (Omega Membrane, 100 kDa Molecular Weight Cut-off (MWCO), Pall Corporation) were compared to other membranes by measuring BSA adsorption. Our use of BSA allowed a valid comparison. For the normalization purposes, the surface area of the membranes was measured by digital imaging. This was done using NIH ImageJ software. In this way, protein adsorption could be normalized to surface area between different sized test membranes. Membranes were then placed in a solution containing unlabeled BSA with tracer I^{125} -labeled BSA. I^{125} -labeled BSA adhering to the membrane was measured using a Packard Cobra-II Autogamma (PerkinElmer Life Sciences, Downers Grove, IL) gamma counter. Background was estimated from tubes exposed to tracer I^{125} -labeled albumin but lacking membranes.

The Omega membranes used for comparison are composed of polyethersulfone (PES), which is also used in many microdialysis catheters. The Omega membrane has been chemically modified to produce an extremely low protein adsorption compared to many other commercially available membranes. The Omega membrane represents a significant advance in reducing membrane protein adsorption. It is the best state of the art commercial membrane currently available. Results of the comparison of SiC to the Omega Pall membrane are shown in Figure 4. The SiC membranes bound a similar amount of BSA as the Pall Omega membrane. The ratio of albumin binding to SiC, compared to the Omega PES membranes, was 0.96 for p-type SiC and 1.12 for n-type SiC.

Conclusion

Free-standing porous SiC membranes were realized using both n- and p-type material and tested to be permeable to proteins and small molecules and capable of separating proteins by molecular weight. Proteins of up to 29000 Da passed through the membrane while those of 45000 Da and higher were excluded. This selective permeability of SiC could be useful in implanted sensors. The low protein adsorption of nanoporous SiC should result in less biofouling. SiC compared very well with polymeric (Pall Corporation Omega) membranes, which have been specifically designed for low protein adsorption. The Omega membranes were at least 3 times better than the previously used best material (regenerated cellulose). From these studies, and others demonstrating the biocompatibility of SiC, porous SiC appears to be a promising material for implantable membranes.

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