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Short communication

Porous silicon biosensor for detection of viruses

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Abstract

There is a growing need for virus sensors with improved sensitivity and dynamic range, for applications including disease diagnosis, pharmaceutical research, agriculture and homeland security. We report here a new method for improving the sensitivity for detection of the bacteriophage virus MS2 using thin films of nanoporous silicon. Porous silicon is an easily fabricated material that has extremely high surface area to volume ratio, making it an ideal platform for surface based sensors. We have developed and evaluated two different methods for covalent bioconjugation of antibodies inside of porous silicon films, and we show that the pore penetration and binding efficiency depend on the wettability of the porous surface. The resulting films were used to selectively capture dye-labeled MS2 viruses from solution, and a viral concentration as low as 2×10^7 plaque-forming units per mL (pfu/mL) was detectable by measuring the fluorescence from the exposed porous silicon film. The system exhibits sensitivity and dynamic range similar to the Luminex liquid array-based assay while outperforming protein micro-array methods.

Keywords: Porous silicon; Biosensor; Fluorescence; MS2; Antibody; Bioconjugation

1. Introduction

Nanometer-scale features are observed in a variety of biological systems, and have evolved to enable a diverse array of functionality. These systems have motivated the development of synthetic, engineered nanoscale systems that could potentially mimic or augment these biological systems. It is therefore important to study the functionalization of such nanoscale materials and their subsequent interaction with biomolecules of comparable dimension. One application that can clearly take advantage of nanometer-scale structures is biosensors. For sensor applications, nanoscale materials provide a large and often highly reactive surface area, which enables more effective capture and detection of molecules than bulk materials. We describe here a new ultrasensitive technique that uses nanoporous silicon to measure small quantities of the bacteriophage virus MS2.

Porous silicon (PS) (Uhlir, 1956) was first developed in the 1950s and exhibits unique optical and electrical properties due to quantum confinement effects (Canham, 1990; Cullis et al., 1997). Porous silicon is easily produced by electrochemical etching in a solution of hydrofluoric (HF) acid, and the pore diameter can be controllably varied from a few nanometers up to several hundred nanometers by adjusting the etching parameters (Herino et al., 1987; DeStefano et al., 2004). After electrochemical etching, the internal pore surfaces of the porous silicon are hydrogen-terminated, which allows one to immobilize large quantities of biomolecules in a relatively small volume through bioconjugation (Mathew and Alocilja, 2005). Consequently, PS can serve as a versatile platform for a biosensor with optical or electrical detection. Silicon is often a material of choice in nanobiodevice design (Borini et al., 2005) as it is biocompatible and readily susceptible to chemical surface derivatization (Buriak, 2002). Here we present our results in developing a prototype fluorescence-based virus sensor using nanoporous silicon.

Viruses are smaller than bacteria and present considerable challenges for detection (Wick and McCubbin, 1999). The bacteriophage virus MS2 was chosen as a model analyte that is often used for simulating biological warfare agents. It is a 27 nm RNA virus that infects *Escherichia coli* male (Stockley et al., 1994).

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We have successfully conjugated a rabbit anti-MS2 antibody inside the PS film using two covalent conjugation protocols. The antibody binding strength and porous layer penetration measurements show that maintaining surface hydrophilicity is critical in achieving high protein concentrations inside the porous material. The sensor selectivity and dynamic range were evaluated using fluorescence measurements with dye labeled bacteriophage MS2 virus. The sensor was shown to be capable of detecting virus concentrations as low as 2×10^7 plaque-forming units per mL (pfu/mL), with three orders of magnitude of dynamic range.

2. Materials and methods

2.1. Porous film preparation

The porous silicon films were prepared using pulsed anodic etching in an HF electrolyte solution (Föll, 1991). The electrolyte solution was held in a cylindrical PVC electrochemical cell, with a platinum sheet as a cathode and a polished p+-doped crystalline silicon wafer as the anode (⟨1 0 0⟩ boron doped, $\rho = 0.5$–$1.0\,\text{m}\Omega\,\text{cm}$, Virginia Semiconductor, VA).[1] The wafer was gently pressed against cell opening through a 1.3 cm diameter Viton O-ring that limited the etched area to $1.32\,\text{cm}^2$. The etching solution was comprised of three parts hydrofluoric acid (48%, J.T. Baker, Phillipsburg, PA) and one part of anhydrous ethanol. Etching was performed using a sequence of current pulses, supplied by a programmable galvanostat (Princeton Applied Research, Model 273A). Each pulse applied a current density of $300\,\text{mA/cm}^2$ to the silicon for a duration of 200 ms, followed by a 10 s pause to allow for equilibration of the HF concentration and the removal of hydrogen bubbles that form inside of the pores during etching.

Porous silicon layers were etched to a depth of either 100 or 1000 nm, by using two different etching times. The porous film thickness was determined by selectively removing the porous layer in a solution of potassium hydroxide and measuring the resulting step-height with a profilometer.

After forming the porous layers, the chips were rinsed in deionized water and ethanol and subsequently blown dry with N_2 gas. Fig. 1 presents a top-down and cross-sectional scanning-electron micrograph of a representative porous film, showing an average pore dimension of 50 nm.

2.2. Bioconjugation chemistry

The electrochemical etching and drying procedure described above results in a porous silicon film in which the internal surfaces are hydrogen terminated (Cullis et al., 1997; Bensliman et al., 2004). Bioconjugation of the porous silicon is accomplished by replacing the hydrogen with a functional organic group that

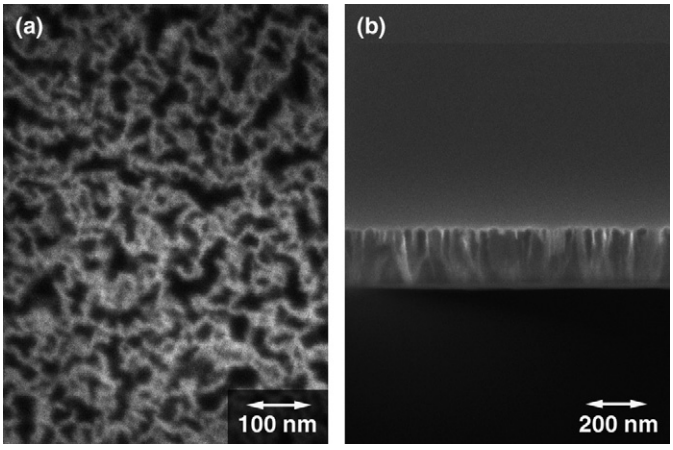


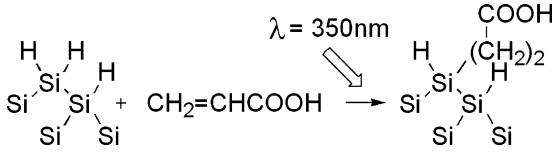
Fig. 1. (a) Top-down and (b) cross-sectional scanning electron micrographs of the porous silicon films produced by electrochemical etching.

can be bound to the desired protein molecule using a cross-linker. We have tested two procedures to make a covalent bond between the rabbit MS2 antibody and the porous silicon surface, one using carbodiimide based protein conjugation and the other based on TDBA-OSu, a photoactivatable aryldiazirine cross-linker. Both procedures have been used in the past for protein immobilization on silicon surfaces (Wang et al., 2004).

For the carboxyl group immobilization the porous silicon sample was put in a quartz cuvette in contact with a 10% volume solution of monomeric acrylic acid in ethanol. The solution was deaerated by means of oxygen-free N_2 flux to prevent the photogenerated radical reaction inhibition and was then exposed to 350 nm light for 1 h in a UV reactor (RMR600, Southern New England Ultraviolet Company, Branford, CT) under continuous nitrogen flux. The silicon samples were then thoroughly washed in ethanol and dried under nitrogen flux. This procedure replaces the silicon surface hydrogen termination with carboxyl terminated functionalities, according to Scheme 1.

The acrylic acid-derivatized porous silicon surface was next immersed in a 0.02 M MES (2-[*N*-morpholino]ethane sulfonic acid) buffer solution (pH 4.8) containing either Alexa 488-labeled or un-labeled anti-MS2 antibodies (0.15 mg/mL), to which 12 μL of 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC) solution in deionized water (10 mg/mL) was added. This reaction was carried out in the dark with gentle mixing for 2 h, followed by rinsing in DI water and nitrogen drying.

For the second functionalization method, an aryldiazirine cross-linker is inserted into the C–H bonds of the methylene groups within the hydrocarbon chain through a highly reactive carbene intermediate (Wang et al., 2004). First, the methylene groups were made available by grafting a hydrocarbon



Scheme 1.

[1] Certain commercial equipment, instruments, materials, or companies are identified in this paper to specify adequately the experimental procedure. In no case does such identification imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are the best available for the purpose.