Communications to the Editor

Identification of Gram Negative Bacteria Using Nanoscale Silicon Microcavities

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Biological sensors fabricated on the nanoscale offer new ways to explore complex biological systems because they are responsive, selective, and inexpensive. Currently, to differentiate Gram-(+) from Gram-(−) bacteria, an ancient staining technique is implemented. In 1884, Hans Christian Joachim Gram at the University of Berlin devised a series of staining steps to differentiate two groups of bacteria based on the structure of their cell walls.[1] For over a century, the Gram stain has been an important tool in clinical analysis, differentiating Gram-(+) bacteria, which remain colored after the staining procedure, and Gram-(−) bacteria, which do not retain the dye. However, this technique is cumbersome, subjective, and prone to human error. We present a new optical approach to detecting Gram-(−) bacteria based on the principles of light interference across multilayers of varying refractive indices. By properly functionalizing the inner surface of a porous silicon layer with highly selective receptor molecules aimed at specific targets, multisensor arrays can be designed to quickly determine the presence of certain pathogens.

Two primary advantages make porous silicon (or nanoscale silicon) an attractive material for biosensing applications. First, its enormous surface area ranges from 90 to 783 m^2/cm^3,[2] which provides numerous sites for many potential species to attach.[3] Second, its eye-detectable, room-temperature luminescence spans the visible spectrum[4] which makes it an effective transducer. Precise control of the nanocrystalline size distribution is extremely difficult; therefore, an alternative method is required to reduce the luminescence bandwidth (typical fwhm ≈ 150 nm). The device nanostructure we have developed, which solves this problem, consists of a microcavity resonator composed of various porous silicon layers, all of which are prepared electrochemically (Figure 1).[5,6] By confining the luminescence generated in the central layer of the microcavity by two Bragg reflectors, the photoluminescence spectrum is composed of multiple sharp and narrow peaks with fwhm values = 3 nm.[7] Upon a refractive index change, the photoluminescent spikes shift, thereby generating a large, detectable differential signal.

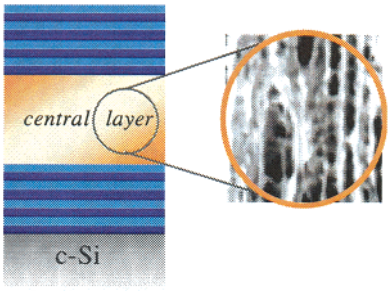
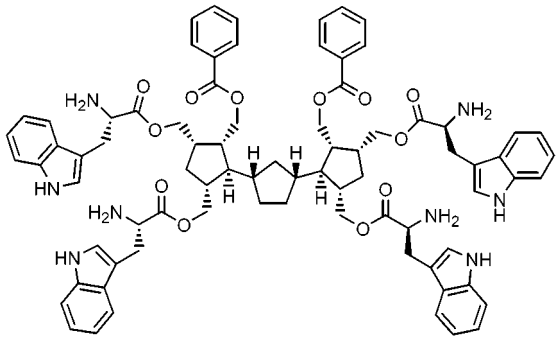


Figure 1. Structure of a porous silicon microcavity resonator. A central active layer of porous silicon is flanked on both sides by a porous silicon multilayer mirror.

In preliminary experiments, we demonstrated the utility of porous silicon microcavity resonators functionalized with oligonucleotides as a means of detecting deoxyribonucleic acid (DNA) sequences, including full-length λ-phage complementary DNA (cDNA).[5,7] Likewise, porous silicon in other configurations has found utility as a base material for the construction of sensors for DNA[5,8−9] and proteins.[8−11]

To specifically detect Gram-(−) bacteria, it was first necessary to select a target molecule present to a significantly greater extent in this bacterial subclass than in Gram-(+) bacteria. Lipopolysaccharide (LPS) is a primary constituent of the outer cellular membrane of Gram-(−) bacteria[12] and is commonly known as bacterial endotoxin, the causative agent of sepsis.[13] The precise structure of LPS varies among bacterial species but is overall composed of three parts: a variable polysaccharide chain, a core sugar, and lipid A. As lipid A is highly conserved among LPS subtypes, this seemed a natural target. We designed and synthesized an organic receptor, tetratryptophan *ter*-cyclo pentane, designated TWTCP, which specifically binds to diphosphoryl lipid A in water with a dissociation constant of 592 nM.[14]



TWTCP

Detailed fabrication steps for the porous silicon microcavity resonator, including the electrochemical preparation and post-

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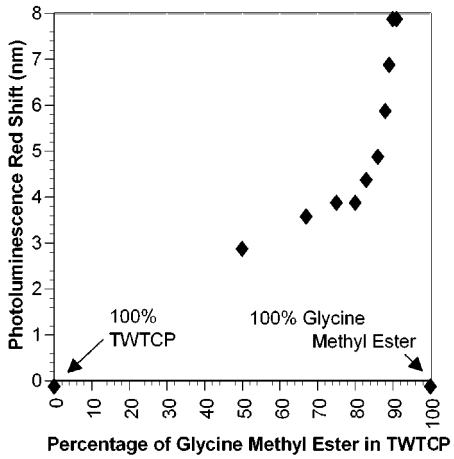

Figure 2. Response of functionalized porous silicon microcavities to lipid A (60 μM) as a function of TWTCP:glycine methyl ester ratio.

thermal oxidation treatment, have been published elsewhere.[5] To provide functionality capable of forming a stable bond between the organic probe molecule and the inorganic silicon surface, the device was first treated with an aqueous solution of 3-glycidoxypropyltrimethoxy silane. Subsequent exposure of this epoxide-terminated surface to an aqueous solution of TWTCP in 6% dimethyl sulfoxide (DMSO), provided a surface functionalized with the lipid A-binding receptor. However, we found in preliminary experiments with purified diphosphoryl lipid A that this procedure did not produce a functional device. We assumed that this was due to all four amino groups of the tetratryptophan receptor reacting with the functionalized porous silicon surface, thus blocking access to the binding face of the receptor molecule. If this were indeed the case, we hypothesized that exposure of the epoxide-terminated surface to a mixture of TWTCP and a "blocking" amine would allow for the generation of a functional sensor.

Using glycine methyl ester as the blocking amine and examining the response of the sensor to purified lipid A, we found that the optimal ratio of receptor to blocker molecules was 1:10 TWTCP:glycine methyl ester (Figure 2). In this case, incubation of the sensor with a solution of lipid A produces an 8 nm red-shift in the photoluminescence peak wavelength. When a 100% solution of TWTCP or a 100% solution of glycine methyl ester is immobilized in the porous matrix, no shifting of the luminescence peaks is detected after exposure to lipid A.

To determine the ability of this sensor to differentiate between two classes of bacteria, independent overnight cultures of Gram-(−) bacteria *(Escherichia coli)* and Gram-(+) bacteria *(Bacillus subtilis*) were grown up, centrifuged, and then individually lysed following resuspension in phosphate buffer solution (PBS, pH 7.4). Upon exposure of the lysed Gram-(−) cells to the immobilized TWTCP biochip, a 4 nm photoluminescence red-shift was detected, shown as the right spectra of Figure 3.[15] However, when the microcavity sensor was exposed to a solution of lysed Gram-(+) bacteria, no shifting of the luminescence peaks was observed, depicted by the left spectra of Figure 3. We attribute the large shift to the recognition and binding of the TWTCP receptor with the lipid A present in the bacterial cell wall. Analogous results were obtained with several other species of Gram-(+) and Gram-(−) bacteria (Table 1). While the sensitivity

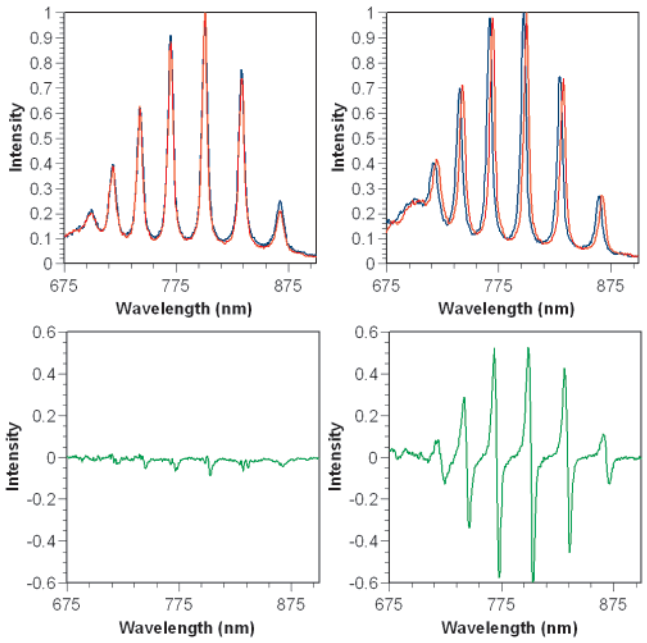

Figure 3. Photoluminescence spectra of a porous silicon microcavity biosensor in the presence and absence of bacterial cell lysates. Blue spectra: sensor alone following derivatization with TWTCP and glycine methyl ester. Red spectra: sensor photoluminescence following incubation with cell lysates from Gram-(+) bacteria (*B. subtilis*, left) or Gram-(−) bacteria (*E. coli*, right). The green spectra in each case are the difference between spectra obtained in the presence and absence of bacterial cell lysates.

Table 1. Response of TWTCP-Functionalized Microcavity Resonators to Bacterial Lysates

bacterium	class	pl red-shift
E. coli	Gram-(−)	4 nm
B. subtilis	Gram-(+)	none detected
L. acidiophilus	Gram-(+)	none detected
Salmonella	Gram-(−)	3 nm
Pseudomonas aeruginosa	Gram-(−)	3 nm

of the device has not been optimized, preliminary experiments suggest that 1.7 μg of bacteria is readily detectable.

These results demonstrate the ability of a porous silicon biosensor to distinguish between Gram-(−) and Gram-(+) bacteria. Clear modulation of the photoluminescence spectra from microcavity device structures illustrates their application as biosensors that can translate the recognition of lipid A present in bacterial cell walls into an optical signal. The remarkable features of these silicon sensors (integratable, high surface-to-volume ratio, robust, inexpensive, small, ease of use) should allow arrays to be constructed to simultaneously identify a variety of analytes by simply functionalizing the surface with arrays of specific, high-affinity receptor molecules. Currently, the sensor is not able to discriminate between different types of Gram-(−) bacteria, but this selectivity issue is a subject under continued investigation.

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Supporting Information Available: Detailed procedure for immobilization of TWTCP and glycine methyl ester on porous silicon, discussion of probable amount immobilized, and discussion of device sensitivity (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(15) We observed that the minimum time needed to red-shift the photoluminescence peaks by 4 nm is 1 h. Prolonged time exposures of up to 5 h show no additional shifting.