Hybrid lipid bilayers in nanostructured silicon: a biomimetic mesoporous scaffold for optical detection of cholera toxin†

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Cholera toxin levels are optically detected by affinity capture within hybrid lipid bilayer membranes formed in the nanostructures of porous silicon photonic crystals.

Porous silicon (PSi), a material with unique structural and optical properties, is produced by anodic etching of semiconductor-grade silicon in ethanolic hydrofluoric acid solutions. The ease with which the porosity, and hence refractive index, can be tailored by changing the current density has enabled the manufacture of complex optical materials, so-called photonic crystals, that exhibit a precise spectral pattern of reflected light.[1] The optical reflectivity profile is dictated by the nanoporous architecture and resulting refractive index of the material.[2] The sensitivity of the reflectivity to changes in the refractive index has enabled PSi photonic crystals to be used as label-free optical transducers for biosensing.[3–10] To use PSi as a biosensor requires a biorecognition interface, composed of specific elements for selective detection of analytes, formed within the porous scaffold.

Biorecognition interfaces on PSi have thus far been limited to immobilization of the biorecognition species to self-assembled monolayers formed from alkyl silanes[5–7,10] or the hydrosilylation of alkenes.[2–4,8,9] Such strategies invariably place the biorecognition molecule in an aqueous environment which is incompatible with many biological systems where multivalent biomolecule interactions occur between species in solution and membrane bound proteins. Hence, the purpose of this communication is to extend the capability of PSi photonic crystals for monitoring biomolecular interactions to those involving lipid bilayers such that dynamic multivalent processes that mimic cell surfaces' transduction of molecular signals can be monitored.

To mimic the natural recognition process of cells, one class of biomimetic lipid interface that shows promise for device construction is the hybrid lipid bilayer membrane (hBLM).[11] hBLMs are formed by vesicle fusion and unrolling onto a hydrophobic surface, such as a non-polar self assembled monolayer (SAM).[12] An important advantage of this interface is the high stability of the underlying surface while maintaining the fluid mobility of the upper lipid monolayer, which is important for reorganization and multivalent binding.

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Herein, we report the formation of hBLMs inside the nanoporous network of a porous silicon (PSi) photonic crystal called a rugate filter (Fig. 1). Porous silicon rugate filters were prepared as described previously[2,8,16] to yield a mesoporous material with an average pore size of 50 nm as shown in the scanning electron micrograph of Fig. 1. A rugate filter has a sinusoidally varying porosity where in the case of Fig. 1 the porosity changes between 62.5 and 64.5%[16] (hence the periodicity in the rugate filter structure is not evident visually). Incident light to the filter results in a narrow high reflectivity resonance in the reflectance spectrum, proportional to the average refractive index of the silicon scaffold and pore voids, at a well defined wavelength. Changes in the reflectivity upon exposure to lipid vesicles are used to assess fusion and unrolling into the porous structure. Confirmation of bilayer formation is commonly assessed using fluorescence and reflectivity methods which are valid for planar biomimetic BLMs but insufficient for three dimensional scaffolds. To date, very few reports of BLMs[13,14] and hBLMs[15] within porous materials have been made and have required free standing membranes and sophisticated instrumentation[15] (*i.e.* neutron source for small-angle scattering) to characterise. In contrast, monitoring the refractive index in photonic crystals with a simple light source as presented here could provide a powerful tool for investigating membrane biology and soft materials at interfaces. Part of the challenge with forming hBLMs in nanoporous media is how to determine that an hBLM has been formed. Here we employ hBLMs composed of lipids containing the glycosphingolipid monosialoganglioside (GM1) that were formed inside rugate filters to monitor the binding of the protein subunit B of cholera toxin. Binding of cholera implies a fluid biorecognition environment indicative of a bilayer as the interaction of GM1 with cholera toxin subunit B requires the reorganization of five GM1 pentasaccharide moieties (Fig. 1, iii).

To form the hydrophobic base that is necessary for lipid deposition and hBLM formation, thermal hydrosilylation of dodecene was performed with the freshly etched filters (Fig. 1, i). Hydrosilylation resulted in a optical red-shift of the filter reflectivity of 24 nm in agreement with expectation, having replaced some of the air in the pores with an organic monolayer (Fig. 2). The quality of the monolayer was confirmed by Fourier transform infrared spectroscopy, showing characteristic organic modes and low levels of silicon dioxide (Fig. ES1, ESI†). Chemically passivated rugate filters resist significant oxidation in air over a six month period and continue to provide a detectable optical signal after two months storage in biological media.[8]

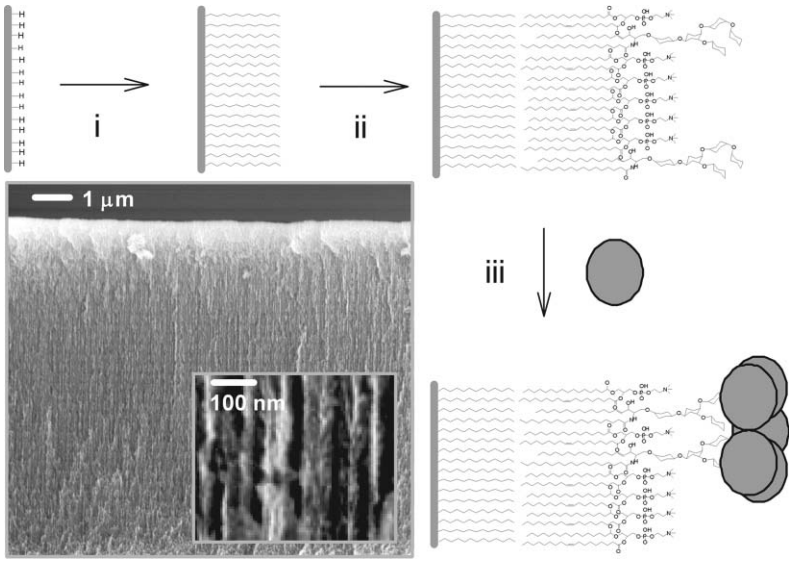

Fig. 1 Scanning electron micrograph of a 60 layer rugate filter, created with a sinusoidally varying refractive index profile normal to the surface during anodization. Scheme for hBLM formation: (i) hydrosilylation of dodecene, (ii) lipid adsorption and bilayer formation and (iii) affinity capture of cholera toxin.

Small unilamellar vesicles (SUV) required for hBLM formation were formed by successive extrusion with a lipid mixture of GM1 and PC (1 : 20) through 0.1, 0.03 and 0.015 μm polycarbonate membranes.‡ Subsequently, hBLMs were self-assembled by incubation of the SUV with dodecene-modified rugate filters, enticing vesicle fusion and unrolling at the pores and into the structure (Fig. 1, ii). Application of lipid vesicles to the modified PSi led to a ~5–10 nm red-shifting of the resonance peak position within one hour. The red shift was significantly larger (by ~15–30 nm) after drying to remove all the water from the pore space of the photonic crystal. Incorporation of another organic layer is expected to yield an additional reflectivity shift comparable to that observed with dodecene. The ~15–30 nm shift observed upon exposure to SUV is similar in magnitude to that observed with dodecene hydrosilylation thus providing evidence for hBLM formation. The use of nanometre filter extrusion during SUV fabrication was essential in forming hBLMs (assessed by magnitude of reflectivity shift). Other vesicle preparation techniques (lipid injection into an aqueous medium) gave comparable results with PC lipids but were insufficient in forming reproducible hBLMs with mixed lipids containing GM1 (no vesicle). Furthermore, the ability of water to penetrate throughout the PSi structure shows the lipids from the SUVs are present throughout the entire photonic crystal. Application of extruded SUVs composed of GM1 and PC gave the same red shift as SUVs prepared from pure PC lipids (Fig. ES2†). The stability of the hBLMs were evaluated by repetitive rinsing cycles and incubations (up to 8 h) with water and PBS. No change in the reflectivity position with these rinsing steps demonstrates the stability of the hydrophobic interaction between the lipid tails and the alkyl monolayer. Importantly for device development, the lipids were easily removed from the mesopores by a brief rinse in ethanol causing a reflectivity blue-shift back to the dodecene modified position (Fig. ES2). The PSi rugate filters could then be reused by simply applying fresh lipid vesicles as before.

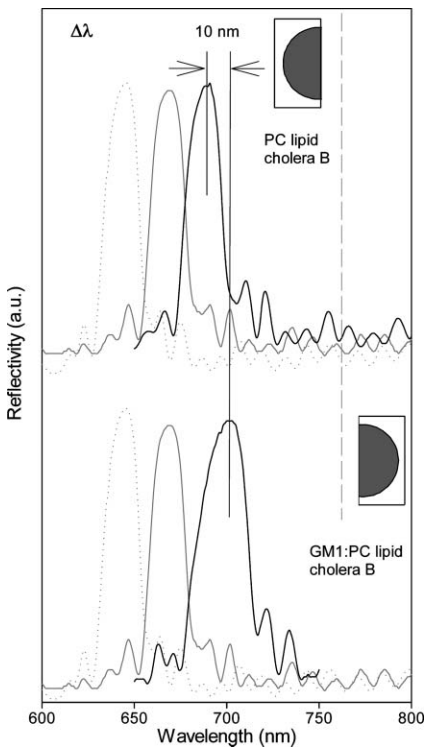

Fig. 2 High reflectivity resonance of porous silicon rugate filters. Top: dashed grey—freshly etched filter resonance, solid grey—dodecane monolayer formation and black trace—phosphotidylcholine hBLM exposed to 0.1 μM cholera. Bottom: same grey spectra as above, black trace—ganglioside GM1 : PC (1 : 20) hBLM after capture of cholera.

Incubation of the PSi with cholera results in red-shifting of the reflectivity spectrum within 4 h at room temperature. Fig. 2 shows the difference between the pure PC hBLM (top) and GM1 : PC hBLM (bottom) after incubation with cholera followed by drying. There is a marked increase in the red-shift, and thus bound cholera, for hBLMs incorporating the cholera-specific ganglioside,

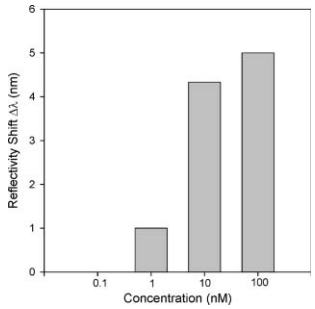

Fig. 3 Observed shift in high reflectivity of ganglioside GM1 hBLMs exposed to different concentrations of cholera toxin.

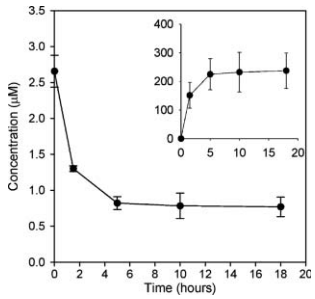

Fig. 4 Drop in concentration of cholera toxin in solution over time in the presence of hBLM PSi as determined by radiolabelled cholera B subunit. Inset: Concentration of cholera within the rugate filters.

indicating successful capture compared with when GM1 was absent. Nonspecific adsorption to the hBLM surface can be compensated for by cleaving the sample in two (as depicted in Fig. 2) and performing a difference measurement between crystals when GM1 is present and absent. The larger observed shift in air is useful for demonstration purposes but optical measurement in aqueous environments is a more likely mode of operation for a practical device. Fig. 3 shows the magnitude of optical response of GM1 presenting hBLMs exposed to sub-micromolar concentrations of cholera, demonstrating a detection limit of 1 nM of cholera which corresponds to 0.2 pmol (in 200 μL volume).

A notable advantage of using porous material for detection of cholera toxin is the high surface area available for binding of analyte. Porous silicon rugate filters have >500 times the surface area of flat substrates of the same planar geometric area rendering them particularly amenable to concentrating a species of interest. Exploiting this quality provides the basis of a new methodology in using photonic crystals for monitoring biomolecular interactions, such that optical detection of the concentration and removal of cholera toxin from a sample in a single step is possible. ^{125}I radiolabelled cholera toxin was monitored in solution over time to evaluate the potential use for this sensor material for sample cleanup. Fig. 4 shows the decrease in concentration of cholera in solution over time with a high starting concentration of cholera in solution (~3 μM). Within 5 h the rugate filter has adsorbed >70% of the toxin. This reduction in solution concentration corresponds to an associated increase within the porous volume of ~100–300 μM (Fig. 4 inset) thus demonstrating the potential in selective capture and concentration of protein toxins.

Using porous silicon photonic crystals to optically detect cholera toxin whilst providing a means for concentration has many advantages. Firstly, porous silicon is fabricated by anodization of semiconductor grade crystalline silicon, providing ease of manufacture. Photonic crystals can be interrogated remotely using simple light sources and no powering of the sensor is required. The simplicity of readout by collecting reflected light may provide a research tool for studying confined biology in anisotropic materials. The huge internal surface area makes concentrating protein toxins a plausible application. Integration of porous silicon photonic crystals into current membrane and filtration systems will enable optical monitoring of cleanup procedures. Furthermore, the mobile lipid layer is easily removed by rinsing with ethanol such that the chemically modified surfaces may be reused a number of times by merely reapplying the appropriate lipid vesicles. Finally, the use of biomimetic hybrid lipid bilayer membranes makes the biosensing method general for detection of any analyte, by merely incorporating the appropriate recognition element within the lipid vesicles.

Exploiting the exquisite recognition properties borrowed from nature with biomimetic interfaces enables the affinity capture and optical detection of cholera toxin. The photonic properties and intrinsic high surface area of porous silicon rugate filters allows for optical detection of toxin capture with simultaneous concentration. Incorporating sensory elements into filtration materials may lead to advanced detection and remediation capabilities, paving the way to new approaches in the battle against disease and bioterrorism.

Notes and references

‡ Lipids were hydrated in phosphate buffered saline and a suspension of large multilamellar vesicles was formed by 4 freeze–thaw cycles and vortexing. This suspension was then extruded to give small unilamellar vesicles.

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