Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics

Journal Name

Dynamic Article Links ►

Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

Supplementary informations

The porous Si layer was fabricated by electrochemical etch of a highly boron-doped monocrystalline (100) silicon substrate (p-type, 1-4 mΩcm), at room temperature with a current density of 85 mA/cm^2 for 100 s in a 2:3 HF(50%):ethanol(99.98%) solution (Labochem reagents); a 1s etch break was applied after 1s of etching, with a resulting ratio of 1:1, to assure a good homogeneity of the electrolyte. In fact, a continuos etching could cause a local depleting of the electrolyte solution, resulting in different etching parameters (porosity and etching rate), and therefore to an inhomogeneous sample.

The chip was thoroughly rinsed with ethanol to remove any HF trace and gently dried under a stream of nitrogen. Before the etch, the silicon wafer underwent a two-steps chemical pre-treatment in order to inhibit the formation of a parasitic surface layer during the etch, as previously reported[15]. In the first step, the wafer was heated in air at 300 °C for 2 h, then exposed to an HF, H2O, ethanol (1 : 1 : 3 volume ratio) solution for 5 min in order to remove the oxide layer formed during the thermal treatment. In a second step, the clean wafer was shortly etched using a current density of 42 mA/cm^2 for 10 s, and exposed to a 0.1 M NaOH solution for two minutes. The chip was exposed to a HF, H2O, ethanol (1 : 1 : 3 volume ratio) solution and then rinsed three times with ethanol and dried under a stream of nitrogen. These conditions produced a pSi film with 65% porosity and a thickness of 4.8 μm about, calculated performing a best-fit calculation of the reflectance spectrum by means of the commercial software SCOUT[18]. From previous characterization data, the produced pSi film is estimated to have pores size ranging from 20 to 30 nm.

In order to substitute the hydrogenated surface of pSi with carboxylic groups to subsequently bind oligomers of chitosan, the hydrosilylation of pSi with undecylenic acid was performed under Argon atmosphere at 130 °C for two and one half hours. The chip was sequently rinsed with ethanol, acetone and ethanol to wash away any undecylenic acid physisorbed on the surface. Crosslinking of the carboxylic acid function present at the surface of porous silicon with amine groups of chitosan was performed using EDC (1-ethyl-3-(3- dimethylaminopropyl)-carbodiimide, Sigma Aldrich) and NHS (N-hydroxysuccinimide, Sigma Aldrich) as coupling agents in a two steps reaction[18]: the coupling solution (6 mg of EDC and 6 mg of NHS in 3 ml of deionized water) was first reacted with pSi for 45 min at room temperature under vacuum (20 torr) to make the diffusion of the solution within the porous matrix easier; the chip was then rinsed with deionized water and dried under a stream of UHP Nitrogen; this first step creates a semi-stable amine-reactive NHS ester, ready for the reaction with a primary amine containing material. The chip was then placed in a solution prepared by dissolving 6 mg of chitosan oligomers (5000 g/mol, Sigma Aldrich) in a 0.16% v/v acetic acid solution (3 ml) stirred for 5h after which the pH was adjusted to 6.9 with the addition of NaOH 0.1M. The reaction lasted overnight under vacuum, to assure a deep penetration of the polysaccharide inside the porous matrix. After the reaction, the pSi sample was thoroughly rinsed with deionized water and gently dried under a stream of nitrogen. Under these experimental conditions it was not necessary to dry the samples in CO2 supercritical conditions as previously reported in Ref. [18]; in fact the lower porosity (higher mechanical strength) and a shorter chitosan oligomer chain with respect to Sciacca et al. didn't cause any collapse of the pSi matrix during the drying step.

2',7'-dichlorofluorescin (DCFH) was synthesized following the procedure reported by Low et al.[20] , starting from 2',7'-dichlorofluorescin diacetate (DCFH-DA) purchased from Sigma Aldrich. The solution was kept in the dark to reduce oxidation to the fluorescent compound 2',7'-dichlorofluorescein (DCF).

Peroxynitrite was synthesized following the method reported by Robison et al.[23]. Three solutions were freshly made in bidistilled water: 0.7M hydrochloric acid+0.6M hydrogen peroxide, 0.6M sodium nitrite and 3M sodium hydroxide, prepared at room temperature. One disposable plastic syringe was filled with the acidified hydrogen peroxide solution, and one with sodium nitrite. A T junction was used to connect the two syringes, and the tubing after the junction was 2 cm long, which assured a proper mixing of the two reagents before the solution arrived in a beaker containing sodium hydroxide stirred vigorously inside an ice bath. The yellow peroxynitrite solution was used once prepared. The concentration of peroxynitrite was measured spectrally at 302 nm, diluting the stock solution with 100 mM NaOH; an extinction coefficient of 1670 M^{-1}cm^{-1} was used to calculate a concentration of 20mM.

The reactivity of the DCF modified pSi towards reducing and oxidizing agents was evaluated by exposing the sample respectively to freshly prepared solutions of glutathione (12 mg/ml, Sigma Aldrich) and peroxynitrite (200 μM, freshly prepared) in PBS, under laser irradiation (λ = 532 nm), and measuring the fluorescence emitted by the pSi chip in real-time. A long pass filter was faced to the collecting fiber to reject the laser light scattered by the porous structure. Figure S1 shows a typical fluorescence spectrum acquired after exposing the chip to the peroxynitrite solution.

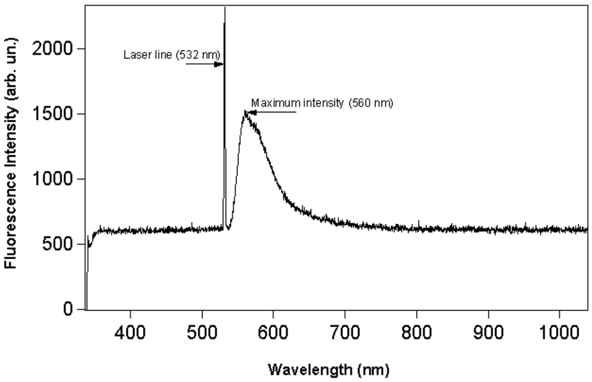

Fig S1: Fluorescence spectrum of oxidized DCF covalently bound to the pSi matrix.