Label-free enzyme biosensor based on porous silicon microcavities

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Herein, we present a porous silicon (PSi) microcavity designed for the immobilization and detection of trypsin enzyme. The extraordinary optical and morphological features of PSi multilayer structures make them an advantageous support for label-free biosensing. Surface modification and enzyme confinement are monitored based on changes in the optical reflectance spectrum. The narrow resonance peak of the microcavity shifts to the red due to an increase in the effective refractive index caused by the attached molecules [1]. Label-free sensors based on optical measurements can be tailored for the detection of molecular binding events. Particularly, the detection of enzymes is of special interest as their levels are indicators of various diseases, such as cancer, diabetes or hyperthension [2,3]. Here, trypsin was selected as a test biomolecule to evaluate the feasibility of the method.

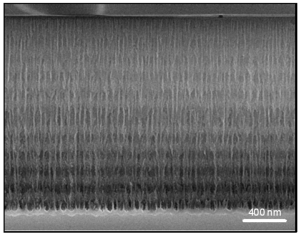

Figure 1: Cross-sectional FESEM of the λ/2 microcavity after oxidation. Each mirror stack is composed by 5 periods of alternating high and low porosity.

A PSi microcavity (PSiMc) is composed of two multilayer λ/4 Bragg mirror stacks (alternating low and high porosity layers) spaced by an active layer (microcavity) [4]. The optical thickness (nd) of the active layer is twice the high-porosity layer (λ/2), where n is the refractive index, d the physical thickness and λ the Bragg wavelength. PSiMc were fabricated by anodic electrochemical etching of a p^{++} silicon (100) wafer (0.002-0.004 Ωcm) using an electrolyte containing hydrofluoric acid (48%), ethanol (98%) and glycerol (98%) in the volumetric ratio 3:7:1 [5]. By alternating a low (25 $mA \cdot cm^{-2}$) and high (70 $mA \cdot cm^{-2}$) current densities, low (65%) and high (80%) porosity layers were obtained. Anodization times of 6.4 s (high porosity) and 11.5 s (low porosity) were chosen to obtain an optical thickness of λ/4 in each layer. The microcavity structure consisted of two 5-period Bragg mirrors and a defect layer of λ/2 optical thickness, for a wavelength range 780-820 nm. After anodization, the microcavities were thermally oxidized at 800 °C for 1 h.

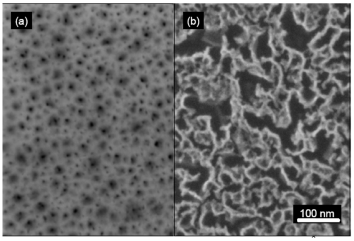

Figure 2: Top view FESEM of the oxidized (a) low (25 mA/cm^2) and (b) high (70 mA/cm^2) porosity layers.

A cross-sectional field emission scanning electron micrograph (FESEM) of the PSiMc (Fig. 1) shows a thickness of 3.7 μm. Fig. 2a and 2b show top-views of the low and high porosity layers after oxidation, respectively. Optical reflectance spectra were recorded using a Perkin Elmer Lambda 950 UV-Vis-NIR spectrometer with a reflection accessory at 15°. The PSiMc spectra before and after oxidation are presented in Fig. 3. The spectrum blue shifted approximately 50 nm due to the formation of silicon oxide, which has lower refractive index.

The surface of the PSiMc was modified using standard silane and glutaraldehyde coupling chemistry to make it suitable for the enzyme

attachment. First, the samples were incubated in 0.5% (3-aminopropyl)triethoxysilane (APTES) solution in toluene at 70 ºC for 2 h. After rinsing with toluene and drying with a N_2 flow, the samples were annealed at 100 °C for 15 min. Following the silanization, the activation of the amine group was performed by incubation in 2.5% glutaraldehyde (GTA) solution in phosphate buffer (PBS).Then the trypsin was immobilized by dipping the samples in 1 mg·mL^{-1} trypsin solution in PBS containing 50 mM benzamidine (to restrict the trypsin autolysis) for 4.5 h at 4 °C. After removing the non-specifically bounded trypsin by rinsing with PBS, the trypsin-immobilized PSiMc was immersed in 1 M tris(hydroxymethyl)aminomethane (Tris-HCl) for 2 h to deplete the residual aldehyde groups.

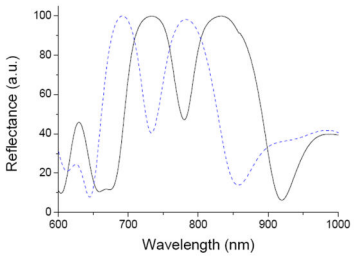

Figure 3: Reflectance spectra of a porous silicon microcavity structure. The solid line correspond sto the as-produced PSiMc and dotted on to the oxidized PSiMc.

The surface modification process (i.e. silanization, activation and enzyme binding) was monitored by recording the optical reflectance spectrum of the PSiMc. The characteristic narrow resonance of the spectrum is very sensitive to changes in the effective optical thickness. The consecutive bindings of the target species cause an increase in the refractive index, revealing a gradual red shift of resonance. The spectrum shift is related to the total amount of analyte immobilized by the PSi structure [1]. Fig. 4 shows how the resonance peak wavelength increases after each functionalization step. The increase of the refractive index after the incubation in APTES and GTA caused a 21 and 18 nm shift, respectively. Finally, after the selective immobilization of trypsin, there was a resonance wavelength increase of 20 nm.

In summary, we proposed a label-free enzymatic biosensor based on porous silicon microcavities. Psi microcavities were designed in porosity, thickness and geometry to obtain the desired optical behaviour. Biological functionalization was performed via silane-gluteraldehyde chemistry to ensure a covalent binding of the enzyme. The optical reflectance spectrum is very sensitive to changes in the effective optical thickness. Thus, the surface modification and the trypsin immobilization were followed by monitoring the optical reflectance before and after the functionalization. The red shift in the spectra confirms the confinement of the enzyme.

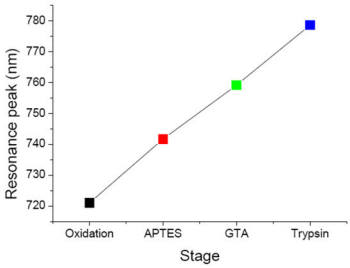

Figure 4: Red shift of the resonance peak wavelength as a result of the different functionalization stages.

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References

[1] H. Ouyang, L. A. DeLouise, B. L. Miller, P. M. Fauchet, Anal. Chem, 79 (2007) 1502.

[2] Y. Shang, W. Zhao, E. Xu, C. Tong, J. Wu, Biosensors and Bioelectronics 25 (2010) 1056.

[3] A. Santos, G. Macías, J. Ferré-Borrull, J. Pallarès, L. F. Marsal, ACS Appl. Mater. Interfaces 4 (2012) 3584.

[4] E. Xifré-Pérez, L. F. Marsal, J. Ferré-Borrull, J. Pallarès, J. Appl. Phys 102 (2007) 063111.

[5] G. Palestino, V. Agarwal, R. Aulombard, E. Pérez. C. Gergely, Langmuir, 24 (2008) 13765.