Oligonucleotides direct synthesis on porous silicon chip

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ABSTRACT

A solid phase oligonucleotide (ON) synthesis on porous silicon (PSi) chip is presented. The prepared Si-OH surface were analyzed by FT-IR and the OH functions were quantified by reaction with 3’-phosphoramidite nucleotide building block. Short ONs were synthesized on the chip surface and the coupling yields evaluated.

INTRODUCTION

The interest in DNA-based diagnostic tests has recently increased since they can be used in a lot of important applications such as gene analysis, fast detection of biological warfare agents, and also in forensic cases. Numerous DNA detection systems, both optical and electrochemical, based on the hybridization between a DNA target and its complementary probe fixed on a solid support have been described.¹ DNA biochips, due to integration and miniaturization of all components, allow continuous, fast and sensitive detection of DNA interactions. The fabrication of a DNA biochip requires the immobilization of the DNA probe on the support surface,¹,² or, as an alternative, the synthesis of the ON strand directly on the device surface.³ -⁵

On the other hand, Porous silicon (PSi) structures are quite ideal support material for chemical and biological sensing, mainly due to their high specific area, up to 600 m²/g, low cost and compatibility with standard integrated circuit processes. PSi is fabricated by the electrochemical etching of a doped silicon wafer in a hydrofluoric aqueous solution. Moreover, its surface can be properly functionalised to covalently link the DNA probe.

In this work, we report the design, fabrication and characterization of a ON biochip based on the PSi nanotechnology for direct DNA solid phase synthesis.

RESULTS AND DISCUSSION

Porous silicon formation and chip assembly. We have designed and fabricated a DNA biochip, just integrating PSi, as the transducer material, and a glass slide, which ensures sealing and the interconnections for fluids inlet and outlet. The silicon wafer used in this work is a p⁺ type, <100> crystal orientation, with a resistivity of 8-12 mΩcm.² The glass is a Borofloat 33 type, 1 mm thick. The reaction microchamber has been realised by a two-step electrochemical etching. The first step is a high current density (800 mA/cm² for about 300 s) electrochemical etch in hydrofluoric acid and ethanol (HF/EtOH 1:1, v/v) solution which creates a μ-well (100 µm deep and a volume of 10 µL) in the bulk silicon. The second step is a consecutive electrochemical etch which is used to fabricate the PSi layer at the bottom of the μ-chamber. The process parameters for this last step were: current density of 400 mA/cm² and etching time of 3.2 s. After the mechanical drilling of the flow channels, the glass slide has been cleaned and activated for the anodic bonding process following standard cleaning procedures. The silicon chip has also been carefully rinsed in deionized water for several minutes. Silicon etched wafer and glass top prefabricated components were anodically bonded together with mutual alignment: a successful bonding, in terms of mechanical strength and bond quality, has been obtained at a temperature of 200 °C, voltage of 2.5 kV, with a process time of 1.5 minutes. The cross section of the chip structure and the general-view of the real device are shown in Figure 1.

Functionalization of porous silicon layer and ON syntheses. The successive treatment of porous silicon layer with a solution of conc. H₂SO₄/H₂O₂ (8:2, v/v, 15 min R.T.) assure the formation of Si-OH groups on the solid matrix. The chip was then, rinsed in deionized water for several minutes and dried under reduced pressure. The FT-IR spectrum (Figure 2) of the chip surface showed the appearance of the characteristic broad bands around 1050
cm⁻¹ (Si-O-Si) and 1065, 880 cm⁻¹ attributable to Si-OH bonds. To determine the surface coverage of Si-OH groups, we reacted the chip 1 with tetrazole activated 5'-DMT-3'-phosphoramidite-timidine nucleotide (2 Scheme 1) which assure the presence of a very reactive phosphorous(III) and

Scheme 1. Solid phase synthesis on PSI-OH chip 1; P: phosphotriester function; R: -(CH₂)₃SO(CH₂)₂; P: phosphodiester function; CE: 2-cyanomethyl; 1: standard ON chain elongation; ii: treatment with methylamine.

a 5'-dimethoxytrityl group (DMT).

The reaction cycle including the standard phosphorous oxidation and the capping step, afforded the PSI-3'-thymidine 4 which was quantified by UV/VIS spectroscopy monitoring 5'-dimethoxytrityl cation released by DCA treatment (ε =71700, λ = 498 nm). The Si-OH functionalization resulted to be in the range of 35-40 nmol/chip and no significative differences were observed varying the concentration of the nucleotide/activator solution (30-60 mg/mL) or prolonging the coupling reaction time (30-180-min). Alternatively, PSI chip 1 was reacted with the phosphoramidite 3 thus obtaining the PSI support 6 (30-40 nmol/chip). The linker 3 ensures a more long and flexible arm for the reaction of terminal alcoholic OH groups and allows the release of the synthesized ON chains by a mild basic treatment, as well. On the supports 4 and 6 three coupling cycles were performed with phosphoramidite 2 thus obtaining PSI supports 5 and 7 respectively. The DMT measurements after each cycle indicated a coupling efficiency of 87-90 % for support 4 and 92-95% for support 6. These findings are in agreement with previous reported ON synthetic procedures on PSI chips which demonstrated the important role of the spacer linker attached on porous silicon surface. The detachment of the synthesized short thymidine oligomer from support 7 was obtained by treatment with anhydrous methylamine (20 min at room temperature). The combined amine solution and washings (methanol and then water), dried under reduced pressure, were analyzed by ESI-MS spectroscopy. The mass data confirmed the presence of expected 3'-phosphate-T₃'-OH product (m/z 931.2 MH⁺). The treatment of 5 with methylamine did not release significative amount of nucleotide material from the chip, also confirmed by DMT deprotection and measurement performed after the amine treatment. FT-IR spectrum (Figure 2) of the PSI-ON chip showed the appearance of the characteristic band around 1100 cm⁻¹ and 860 cm⁻¹ attributable, respectively, at P=O and 5'-CH₂-OH.

CONCLUSION

In this preliminary experiments of ON synthesis we have tested the direct reactivity of the freshly prepared PSI OH functionalized chips with standard phosphoramidite building block and confirmed the compatibility of the PSI-3'-bonded thymidine with the chemical treatments required by standard ON solid phase synthesis. The synthesis of support 4 allowed as to estimate the amount of accessible OH groups on the silicon surface and to obtain a reproducible nucleotide functionalized chip. The data were also confirmed by the synthesis of the PSI-support 6. We hypothesize that, after coupling yield optimization, the support types 4 and 6 could be exploited both to obtain deprotected ON bonded to a PSI chip and to achieve a standard ON solid phase synthesis on PSI matrix.

REFERENCES


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