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Stepwise Growth of Oligodeoxynucleotides on Solid Hydroxylated Substrates: Characterization of the Growth by UV-Vis Spectroscopy and Ellipsometry

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A stepwise growth of oligonucleotides on silicon wafer and fused silica is described. Spectroscopic analysis such as ellipsometry and UV-Vis spectroscopy, and contact angle measurement after each synthetic step have confirmed that a 15-mer oligonucleotide was successfully grown on the hexaethylene glycol linker bound to a silicon wafer or fused silica via a glycidoxypropylsilane.

Keywords DNA chip; stepwise DNA synthesis

INTRODUCTION

DNA chips are glass surfaces that represent thousands of DNA fragments arrayed at discrete sites. These DNA-modified surfaces are the subject of considerable current activity in the field of biotechnology that holds great promise for identifying gene polymorphisms that predispose man to disease, gene regulation events involved in disease progression, and more-effective disease treatments. Despite their growing importance, several aspects of the performance of these novel composite materials are far from the optimum, and their surface chemistry remains poorly characterized. Southern et al.[1] and other workers[2] have reported that oligodeoxynucleotide can be successfully synthesized on glass substrates such as CPG (controlled pore size glass) and glass slides. However, the surface properties during the surface

modifications were not characterized spectroscopically. In order to characterize the chemistry of DNA-modified surfaces unambiguously, UV-vis spectrophotometer, ellipsometer, and contact angle goniometer measurement were utilized.

EXPERIMENTAL

Clean silicon wafer and fused silica were immersed in a toluene (20 ml) solution of 3′-glycidoxypropyltrimethoxysilane (0.2 ml). The solution was kept at room temperature for 6 hours under nitrogen. The resulting substrates were baked at 120 °C for 30 min and sonicated in toluene and methanol for 10 min respectively, and thoroughly washed with methanol. Finally, the washed substrates were dried *in vacuo* at room temperature.

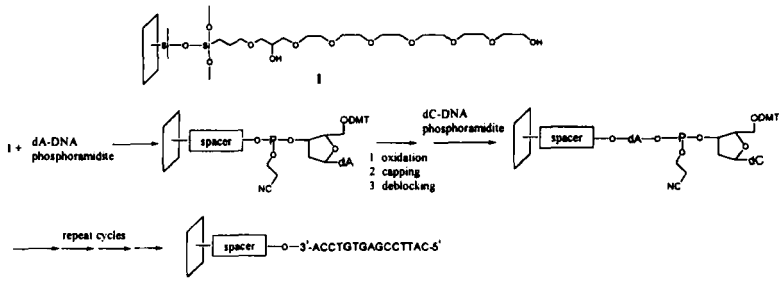
The silylated substrates were immersed in a CH_3CN (10 ml) solution of hexaethylene glycol with a catalytic amount of *p*-toluenesulfonic acid. Subsequently, toluene (10 ml) was added as a cosolvent. The solution was heated to 80 °C for 12 hours and then the substrates were washed thoroughly with toluene and methanol and dried *in vacuo*.

The substrates were immersed in a CH_3CN (20 ml) solution of DNA phosphoramidite (50 mg) and activator (tetrazole), followed by oxidation, capping, and deblocking [3].

RESULTS AND DISCUSSION

It is observed that hexaethylene glycol linker, bound to the glass via a glycidoxypropylsilane, terminating in a primary hydroxyl group is satisfactory as a starting point for oligonucleotide synthesis (**1**). The thickness of a modified surface is 20 (± 2) Å and a 15-mer oligonucleotide was synthesized on the surface by repeating the coupling, oxidation, capping, and deblocking steps in a cyclic manner

(SCHEME 1). The thickness was measured by ellipsometry using refractive indices that were set for organic materials as default. The increase of thickness per DNA monomer on the linker is about 3 Å in



SCHEME 1 Stepwise synthesis of 15-mer ODN. The target sequence of 15-mer ODN is 3′-ACCTGTGAGCCTTAC-5′.

the early stage, but decreased slowly as the ODN chain lengthened so that the increase of the ODN layer is not linear (FIGURE 1(a)). This could be partly due to a conversion yield lower than 100 %. Also, rearrangement of the chain to fill vacancies might contribute the deviation from the linearity.

We can also follow the each reaction step directly by UV-vis spectrometry. After each coupling step, two peaks (240 nm and 255 ~ 285 nm) are observed. The former originated from the blocking group

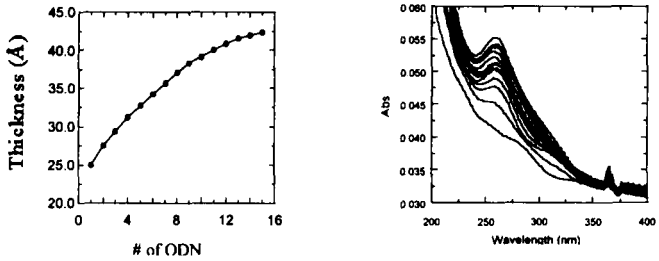


FIGURE 1 (a) Thickness estimated by ellipsometer (b) UV-vis spectra of 1-15mer. Spectra were taken after each deblocking step.

(-DMT) of 5'-OH and the latter from the base of DNA. The former totally disappeared after each deblocking step (FIGURE 1(b)). The continuous increment of absorbance confirms that the 15-mer ODN grows successfully on the substrates.

Contact angle measurement also can give useful information about the properties of surface end groups. The static contact angles that are measured after each step of synthesis mode are different from each other, which mean that the end group of the surface is changed step by step (TABLE 1). UV-vis spectra, contact angles, as well as thickness increment confirmed that the 15-mer oligonucleotides were successfully synthesized on silicon wafer and fused silica.

	Contact angle (°)
after cleaning	< 10
after linker attachment	55 - 59
after coupling	72 - 75
after deblocking	60 - 65

TABLE 1 Water contact angles of each step of synthesis. The values are measured in the static mode.

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