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Using Silane Coupling Reagents in Fabrication of DNA Arrays on Glass Support

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Two silane reagents were used to modify the surface of glass slide. The modified surfaces were characterized using X-ray photoelectron spectroscopy (XPS). DNA arrays generated by polymerase chain reaction (PCR) from the genomic DNA of hepatitis B virus (HBV) were spotted on the slides by capillary tube. Such slides were applied to detect the HBV DNA fragment by hybridization with fluorescence-labeled probe.

Keywords: silane; self-assembly; DNA array; hepatitis B virus

INTRODUCTION

In recent years the organized molecular systems constructed by self-assembly method have been used in many areas such as molecular electronics, nonlinear optics and biology. The 'chip-based' approach utilizes DNA arrays immobilized on glass as targets. Fluorescent probe hybridizes to the arrays allowing detection of DNA fragment interested [1]. In this paper we report the preparation of monolayer film constructed via Si-O coupling reaction. A useful and simple method for fabricating the DNA arrays on the modified surface of glass support has also been developed. Two silane reagents were used to modify the slide surface on which we immobilized the DNA arrays. We have put such arrays into preliminary use of hybridization detection between the DNA fragment

fragment and fluorescence-labeled probe.

Using two silane reagents, γ-aminopropyl triethoxy silane (APTES) and β -aminoethyl-γ-aminopropyltrimethoxysilane (AEAPTES), we modified the surface of the glass substrate and immobilized the DNA fragment produced from PCR(polymerase chain reaction) to generate the DNA arrays, which were then used to detect the HBV (hepatitis B virus) DNA by fluorescence-labeled probe.

The approach involves the modification of glass surface by silane, the fabrication of the arrays and the fluorescence detection of HBV DNA based on hybridization.

EXPERIMENTAL

Self-Assembly of Silane Coupling Reagents on Glass Support

The glass support consisting of the 7.5 × 2.5 cm slides were prepared by cleaning in washing liquid, rinsing 1 mol/L NaOH , exhaustive washing in distilled water. The slides were then immersed for 2hr with a solution of 10%(vol/vol) silane (Nanjing Shuguang Chemical Factory) in ethanol for 2hr and then, rinsed thoroughly with ethanol and dried at 50°C, heated at 100°C for 15 min. Such modified slides were characterized by X-ray photoelectron spectra (XPS). A V.G. Escalab MK II system was used to record the XPS spectra and to determine the surface composition of the glass support.

Preparation of Arrays and Detection of Hybridization

The 121bp DNA fragment specific for HBV surface antigen (HBsAg) was amplified by PCR from HBV genomic DNA which was prepared from the serum of patients who have hepatitis B. The DNA arrays were fabricated on the modified slides. The capillary tube loaded ~0.02 μl of the concentrated PCR product onto the surface of glass. Adjacent samples were spotted ~200 μm apart. After the spotting operation was complete, the slides were rehydrated in a humid chamber for 2hr, baked in an 80°C oven for 2hr, then rinsed in 0.1% sodium dodecyl sulfate (SDS) and double distilled water, then dried in the air. The spotted arrays were denatured in a 90°C water bath for 2min and then chilly cooled on ice water. The 5'-fluorescence probe consisting of 42bp single-strand DNA labeled by fluorescence -1-phosphoramidite (FAM, Sangon Co.) was added to a final concentration of 1 μ mol/L in 5×SSC /0.1%SDS. The entire 40μl of probe

solution was transferred to the array surface, covered with a coverslip, and incubated in a 55 °C water bath for 1hr. Then the slides were washed in 2 ×SSC, 0.3×SSC at 37℃ for 5min, two times respectively. Finally, the hybridization pattern was observed by fluorescence microscope.

RESULTS AND DISCUSSION

XPS is a highly diagnostic tool for the assessment of the chemical state of element. The binding energy scale was calibrated to 285.0eV for the C_{1s} feature. XPS spectra of glass surface modified by two silane reagents see Figure 1.

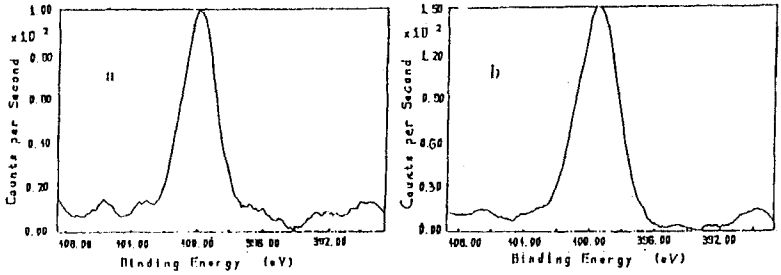

FIGURE 1 XPS spectra in the N1s of: (a) APTES film, (b) AEAPTES film

In fig.1a, N1s has only one feature at 400.0 eV which corresponds to the N1s of NH_2. Fig. 1b shows that N1s has the feature at 399.7eV. These values are slightly greater than the 398.8 expected from the reference [2], which we attribute to the presence of Si-O bond. Since there is no nitrogen element on the slides before modification, above XPS spectra shows that the silane layer had been deposited on the substrate. The data of quantification by XPS are shown in Table Ⅰ.

TABLE Ⅰ The data of quantification by XPS

Region	BE (eV)		Raw area(CPS)		Atomic %		Stoic	
	a	b	a	b	a	b	a	b
C1s	285.0, 285.0		1752, 1659		37.84, 51.63		1.00, 1.00	
O1s	532.4, 532.1		4790, 2390		44.65, 31.92		1.18, 0.62	
N1s	400.0, 399.7		279, 454		3.80, 8.86		0.10, 0.17	
Si1s	103.1, 102.5		740, 296		13.70, 7.85		0.36, 0.15	

a: APTES, b: AEAPTES

From Table I it is seen that the atomic percentage of nitrogen element of AEAPTES is larger than that of APTES, which corresponds to its chemical component.

DNA arrays of HBsAg with about 200 μm feature size have been detected successfully in pilot experiment. Total image scheme and the result of hybridization under the microscope are shown in Figure 2.

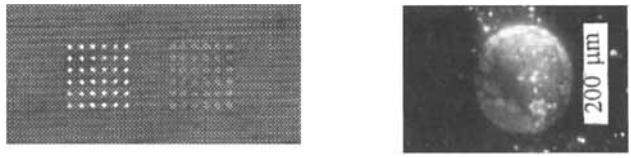

FIGURE 2 (a) Scheme of detection for hybridization, the left array shows positive results, the right array shows negative control,
(b) Fluorescence image of the spots on slide modified by APTES
See color plate XVI at the back of this issue.

We have fabricated the DNA arrays on the surface of glass using two silane reagents to modify the slide. The simple arrays used in the present study were prepared manually. The construction of substantially more complex arrays could be accomplished with robotic [3] or photolithography methodologies. Detection limit is ~2pmol using the fluorescence-labeled probe (detail data not show).

As we know, although the advent of PCR has made possible the rapid and simple amplification of a target sequence of interest, the need for manual and laborious gel-based methods for analyzing PCR results has compromised its utility for the routine detection of pathogen DNA. Our initial motivation for developing these arrays arose from the need for detection of PCR product so we should immobilize the oligonucleotide on the support, and apply the color PCR to amplify the target DNA fragment. Experiments including clinical application are in progress to explore the feasibility of such arrays.

References

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