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A Novel Substrate for *in situ* Synthesis of Oligonucleotides: Hydrolyzed Microporous Polyamide-6 Membrane

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Abstract: A novel substrate for *in situ* synthesis of oligonucleotide was prepared by hydrolyzing microporous polyamide-6 membranes in a 0.01mol/L NaOH/(H_2O-CH_3OH) mixture medium. The formation of amines (NH_2) on the surface was proved by attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR). The treated membrane was applied for *in situ* synthesis of oligonucleotide and a single step coupling efficiency determined by ultraviolet (UV) spectra was above 98 %.

Keywords: Catalyzed hydrolysis, *in situ* synthesis of oligonucleotides, coupling efficiency, polyamide-6 membrane.

DNA microarrays, also known as DNA chips or gene-chips, is attracting more and more attention from scientists. It actually comprises of an array of complementary DNA (c-DNA) or the synthesized oligonucleotide probes immobilized by spotting or *in situ* synthesis on a solid substrate such as glass slide, organic polymer film, silicon wafer, and so on. Among these substrates the modified glass slides is almost the only one kind of substrate that has been widely used as substrate for the *in situ* synthesis approach[1]. Microporous membranes such as nylon, polypropylene are mainly used for the spotting method and detected by means of [γ-^{32}P]ATP labels[2-5] because of the strong fluorescent background. Therefore, it seems that these membranes are not excellent substrates for gene-chips. However, with the development of some new methods such as the gold-labeled silver staining[6-8] and time-resolved fluorometry[9-13], to overcome the fluorescence noise arising from the substrates have come to us. Since organic polymers are easily processed and modified, we can greatly expand the substrate availability for gene-chips by modifying organic polymers and applying new bio-molecular labeling and probing methods. Here we provide a new method to prepare the gene-chip substrate by hydrolyzing the microporous polyamide-6 membranes in a 0.01 mol/LNaOH/CH_3OH solution medium under reflux.

The polyamide-6 microporous membrane (50 mm in diameter, 0.2~0.5 µm in average aperture) was obtained from Biochemical & Plastic Corporation of Taizhou, China. For the hydrolysis modification of the membrane, 90 mL of 0.01 mol/L sodium hydroxide

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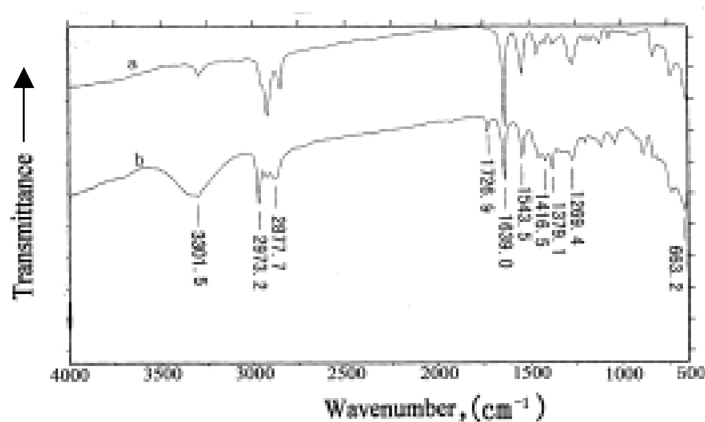
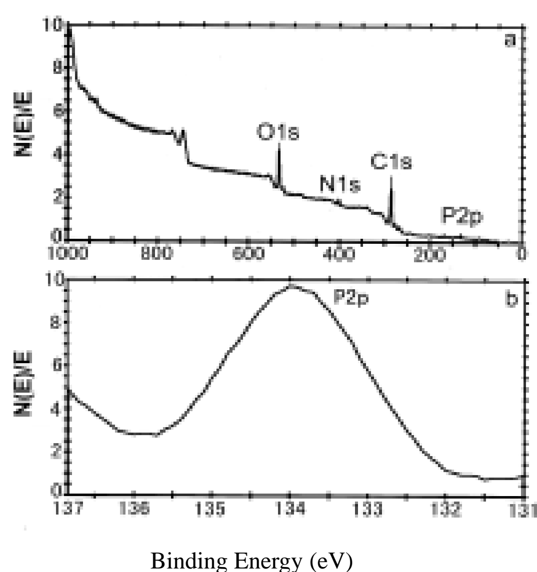
solution and 90 mL of methanol were added to a 250 mL flask equipped with a thermometer. The polyamide-6 microporous membrane segments (about 1cm2) were then immerged into the mixture solution, and then hydrolyzed for 36 h at refluxing temperature 353 K to cleave a portion of amides (-CO-NH-). After being cleaned ultrasonically, washed fully to neutrality with distilled water and dried under vacuum, the treated membranes were submitted to the characterization of attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) on the Nicolet Nexus 870 FTIR instrument with a DTGS detector at a resolution of 4 cm^{-1} and 64 scans.

Figure 1 ATR-FTIR spectra of nylon-6 microporous membrane surface



a) before hydrolysis, b) after hydrolysis.

Figure 2 XPS spectra of nylon-6 microporous membrane



Binding Energy (eV)

a) after *in situ* oligonucleotide synthesis, b) the P2p core-level spectrum after *in situ* oligonucleotide synthesis.

Figure 1 shows the ATR-FTIR spectra of the nylon-6 microporous membarne surface before (**a**) and after (**b**) hydrolysis. From **Figure 1a**, one can find that the unhydrolyzed microporous membrane exhibits no absorbance at 1726.9 cm^{-1}. However, this band is present in **Figure 1b**. This band is related to the C=O stretching vibration of carboxyls. The above result demonstrates that the hydrolysis treatment has given rise to the formation of carboxylates. It is also shown that the broad band at 3301.5 cm^{-1}, usually assigned to the N-H bending vibration in primary amine, is obviously enhanced. However, the absorbance at 1639.0 cm^{-1} related to C=O of amides and the combination absorbance of the N-H and C-N of amides at 1543.5 cm^{-1} are decreased. These changes clearly indicate that the amount of amides (-CO-NH-) on the surface of nylon membrane has been decreased and meanwhile a lot of amine ($-NH_2$) groups were produced. The later makes it possible to synthesize oligonucleotide *in situ* on the as-obtained nylon-6 membrane.

The automatic oligonucleotide synthesis (5′-NH_2-AAC CAC CAA ACA CAC-3′) on the modified polyamide-6 microporous membranes was conducted as previously described according to the standard phosphoramidites chemistry protocol[14], except that the microporous membrane (1 cm^2) was placed into the closed sandwich-like fluidic reactor system which was jointed to the Model 391 DNA synthesizer. After the oligonucleotide synthesis, the membranes were further characterized with X-ray photoelectron spectra (XPS) on the ESCA LAB MK2 instrument with Mg K-Alpha as source and the C (1s) level (284.4 eV) as the reference binding energy under a pressure of 1.33×10^{-7} Pa. **Figure 2** shows the XPS spectra of nylon-6 microporous membrane after (**a**) the *in situ* synthesis of oligonucleotide probes, and the P2p core-level spectrum of nylon-6 microporous membarne after the *in situ* synthesis of oligonucleotide probes (**b**). Three peaks having binding energies at about 285 eV, 400 eV and 530 eV can be observed in **Figure 2a**, they are ascribed to C1s, N1s and O1s, respectively. They were also be observed when the membrane was not treated. Besides, the above three bind energy peaks, the peak having binding energy at 134.2 eV relative to P2p of P=O can also be observed in **Figure 2a**. This observation clearly indicates the existence of P atoms in the surfaces of the membrane on which the *in situ* synthesis of oligonucleotide was performed, which is further verified more clearly in **Figure 2b**, where the 134.2 eV band attributed to P2p of P=O of phosphoric groups is very apparent, indicating the successful synthesis of the oligonucleotide probes.

It is known that if a mole of nucleoside monomers (dA, dT, dC and dG) were coupled to the treated membrane or the previously synthesized oligonucleotide, a mole of dimethoxytrity (DMT), which is used to protect the 5′-hydroxide (5′-OH) group of the four kinds of nucleoside monomers (dA, dT, dC and dG), will be eliminated at the next deprotection step. The thin solution of DMT accords with the Beer's Law and displays a highest absorption peak at the wavelength of 498 nm which is unique to DMT. Therefore, by checking the absorbance of DMT (on a Shimadzu UV-2201 spectrophotometer) for each coupling step, we can know the coupling efficiency of each addition step. It was demonstrated that an average coupling yield of more than 98% was achieved on the modified membrane, which is true for the *in situ* synthesis of DNA. Our previous study

indicated that the coupling efficiency of prime 2-7 bases ranged from 88% to 93% on modified glass slides in a procedure reported here[14], and then rose to 98%, which is in accordance with that reported by Affymetrix corporation but is obviously lower than the coupling efficiency of our modified nylon-6 membranes reported here. Moreover, in experiment we also observed that the solution of DMT for hydrolysis made the membrane much darker than that for the modified glass slide with the same area which is widely used as a substrate for the commercially available bio-chips at present. It is suggested that the probe density in the hydrolysis treated microporous nylon-6 membrane is much greater than that on a modified glass slide. We believe that the hydrolysis microporous nylon-6 membrane could lead to the eventual development of a novel substrate for the *in situ* synthesis of DNA microarrays. The detailed investigation of this modified membrane and its application will be reported elsewhere.

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