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In situ Synthesis of Oligonucleotides on Plasma Treated Polypropylene Microporous Membrane

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Abstract: Polypropylene microporous membranes were treated with plasma in a mixture of N_2 and H_2 (1:2 in volume). Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR), X-ray photoelectron spectroscopy (XPS) and ultra-violet (UV) spectra demonstrated the success of grafting amino groups. The density of the polar amino groups on the membrane surface is about 0.59 μmol/cm^2. The as-treated membranes were successively applied to the *in situ* synthesis of oligonucleotides and an average coupling yield was more than 98%. The surface feature of the treated membrane is suggested to be responsible for its advantage over a glass slide.

Keywords: Plasma, grafting, amino group, *in situ* synthesis of oligonucleotides, coupling efficiency, polypropylene microporous membrane.

Nowadays much attention has been focused on highly parallel DNA hybridization assays on miniaturized gene-chips[1]. The substrates for *in situ* synthesis of DNA microarray are usually glass slides developed by Southern *et al.*[2], or silicon wafers. However, when fabricating a gene-chip by means of *in situ* synthesis[3], there is limitation to the probe density and coupling efficiency, so better substrate materials remain to be discovered[2]. Recently, plasma treatment has been used to alter the surface properties of polymers without changing their bulk properties[4-8]. We here report the successful grafting of $-NH_2$ on a polypropylene microprous membrane by simple plasma-treatment in a mixture of nitrogen(N_2) and hydrogen(H_2). The modified microporous polypropylene membrane serves well as a substrate for the *in situ* synthesis of oligonucleotides and an average coupling yield was more than 98%.

The polypropylene microporous membrane (diameter=47 mm, average aperture= 0.45 μm, named as membrane A in this paper) and the reference porous polypropylene membrane (AP01-81, designated as membrane B in this paper) were obtained from Germany Pall Corporation. The latter was polyacrylamide modified with PEG (poly (ethylene glycol)) spacer, loading with free primary amino functions (0.6 μmol/cm^2).

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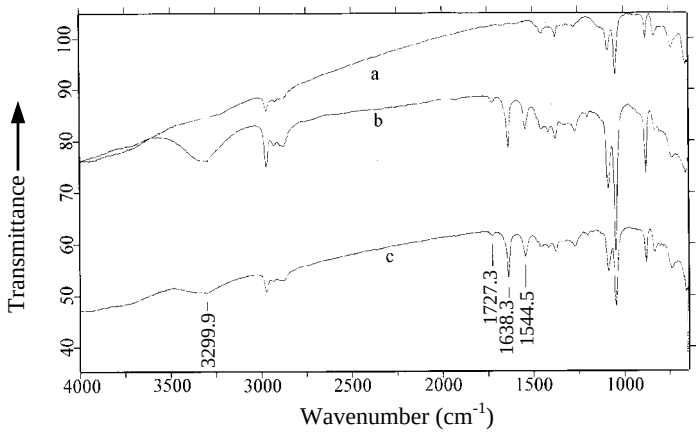
The commercially available ultra-high pure gases (purity of $N_2 \geqslant 99.9\%$; $H_2 \geqslant 99.99\%$) were used without further purification. Generally, by means of the interaction of plasma on the polymer surfaces in mixture of N_2 and H_2, the structures of the membranes were changed and grafted with amino groups. Our typical treatment condition is: discharge power of 80 W, treatment time of 120s and vacuum of 2 Pa. The fluxes of N_2 and H_2 were 30 mL/min and 60 mL/min, respectively. The gases were introduced through flow controllers and the pressure was about 150 Pa.

As shown in **Figure 1a** for the 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., it is found that the untreated membrane A exhibits no absorbance at 3299.9, 1727.3, 1638.3 and 1544.5 cm^{-1}. However, after the plasma treatment all the above four bands are present in **Figure 1b** and **c** (**b**: face, **c**: inverse). The band 1638.3 cm^{-1} is usually assigned to the N-H bending vibration in primary amine and amide ($R\text{-}NH_2$, R-CONHR), and the band 1544.5 cm^{-1} to the N-H deformation, the N-H bending and the C-N stretching vibration in secondary amine (R-NHR). The weak absorbance band at 1727.3 cm^{-1} is related to the polar carbonyls and the broad band with a maximum at ~3299.9 cm^{-1} to amine and amide ($R\text{-}NH_2$, R-CONH). The above observation consists with that reported previously[8-9], demonstrating the plasma treatment leads to the grafting of amino groups and others amides. Moreover, it seems that the intensity of the absorbance for the inverse side (**Figure 1c**) is slightly weaker than that for the face (**Figure 1b**), meaning a possible difference in bombarding strength of plasma action for both surfaces. This was taken into consideration of performing characterization and *in situ* oligonucleotide synthesis in our investigation.

The above plasma grafting was further verified by XPS analysis of the treated membrane A. Three peaks having binding energies at about 285 eV, 400 eV and 530 eV were observed[10], where are ascribed to C1s, N1s and O1s, respectively, clearly indicating the existence of N atoms in the treated surface. The N1s photoelectron peak

Figure 1 ATR-FTIR spectra of the membrane A surface



(a) before plasma treatment, (b) and (c) after plasma treatment (b: face, c: inverse).

of the modified membrane A (not shown here) was deconvoluted into two components by peak fitting. The first at 398.57 eV is attributed to –N–C nitrogen (80.82%) and the second at 400.25 eV can be attributed to imine (C=N) or amide (–N–C=O) (19.18%)[11-13], verifying the success of plasma grafting of groups associated with N for membrane A.

The plasma modified membrane A was then submitted to automatic oligonucleotide synthesis (5′-NH_2-AAC CAC CAA ACA CAC-3′) as previously described according to the standard phosphoramidites chemistry protocol[3], except that the modified membrane A (1 cm^2) was placed into the closed sandwich-like fluidic reactor system jointed to the Model 391 DNA synthesizer. 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 nucleoside monomers, will be eliminated at the next deprotection step. The thin solution of DMT displays a highest absorption at 498 nm according with the Beer's Law. Checking the absorbance of DMT of four parallel tests (Shimadzu UV-2201 spectrophotometer), an average coupling yield was more than 98% for each step and it is very true for *in situ* synthesis of DNA. Our previous study indicated that the coupling efficiency of the prime 2-6 bases was in the range of 88% ~ 93% on a modified glass slides under the same *in situ* synthesis procedure, and then rose to 98%[3], lower than that of the present plasma treated membrane A. Moreover, we also observed that the solution of DMT for the plasma modified membrane A looked similar to that for the reference membrane B, but much darker than that for commercially available modified glass slide with the same area. From the absorbance of DMT solu-

Figure 2 The arrangement of the functional groups or the previously synthesized oligonucleotide on glass slide (A) and a fraction of plasma modified membrane A (B)

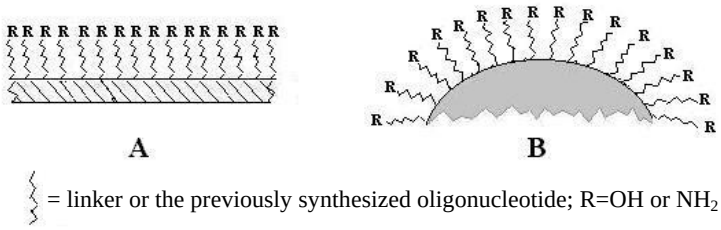
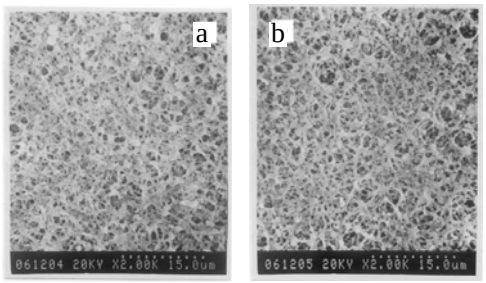


Figure 3 SEM pictures of polypropylene microporous membrane A



(a) before plasma treatment, (b) after plasma treatment.

tion from the first attached base, the density of the active amino groups on the surface of the plasma modified membrane A is about 0.59 μmol/cm^2.

The advantage of the plasma treated membrane A over the glass slide could be attributed to its surface structure. The surface of a glass slide is smooth, the growth of the oligonucleotide chain will initiate from functional groups to distribute on the functionalized surface in two dimensions (**Figure 2A**). However, the scanning electron microscopy (SEM, Hitachi X-650) image in **Figure 3** displays that there are more pores in nanometric or micron size present in the plasma modified membrane A than those in the untreated membrane, and the whole membrane looks like a web-like structure consisting of some "threads" and the BET (Barret-Joyner-Halenda) surface area increased from 0.9097 to 6.8371 m^2/g (N_2 adsorption data at 77 K, Micromeritics ASAP 2010). The oligonucleotide chain is believed to initiate from the functional group to distribute on the surface of the modified membrane A in three dimensions. It has been known that there is an effect of steric crowding on the interaction between substrate and probe, and on the hybridization of target with probe[2]. As shown in **Figure 2B**, the functional groups covalently bonded to the extrusion surface of the treated membrane provided more available space for the coming nucleoside monomers and made the nucleoside monomers react with the functional groups or the previously synthesized oligonucleotide more easily and coupling efficiency for the prime 2-6 bases could be higher.

From all the above, the plasma modified membrane A serves well as a substrate for the *in situ* synthesis of DNA microarrays. The investigation of the mechanism about the plasma grafting and the application of the plasma modified membrane A are in progress.

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