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Development of oligonucleotide microarray involving plasma polymerized acrylic acid

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ABSTRACT

This paper presents the manufacturing of biochips by using the COOH– derived polymer coating deposited by plasma polymerization of acrylic acid. This technology is based on depositing a thin layer obtained by plasma polymerization of acrylic acid which allows a further covalent immobilization of biomolecules on glass substrates. The plasma power value was optimized to maximize the stability of plasma polymerized acrylic acid (PPAA) coatings in water, which has a very important role for such applications. In order to obtain a covalent immobilization of DNA probes on the PPAA coated surface, the activation protocol of carboxylic function was carried out with the help of N-Hydroxy Succinimide and 1-Ethyl-3-(3-DimethylAminopropyl) Carbodiimide. The efficiency of PPAA coated in microarray applications was compared with two types of commercial slides. Such surfaces have shown very interesting results in terms of relative density of attached DNA probe molecules and signal-to-background ratio measured for target DNA hybridization. Nonspecific DNA bonding measurements showed only a small amount of nonspecific physisorption between the DNA probe and the PPAA-activated surfaces. This work shows that the plasma polymerization technique can be successfully applied to produce a high-quality glass surface for the manufacturing of DNA arrays.

1. Introduction

The development of high-density oligonucleotide arrays or "DNA chips" has revolutionized the fields of genomics [1,2]. A microarray is an orderly arrangement of DNA probe molecules immobilized at micro-scale locations on a solid substrate such as glass, nylon or silicon substrates. Hybridization of complementary targets to these arrays allows the deduction of information such as DNA sequences, drug treatment strategies via differential genes expression monitoring, diagnostic tests, and patient drug response [3,4]. The great advantages of microarray technology are based on a high-throughput and reproducible analysis of thousand of genes.

Two main strategies have been developed for oligonucleotide arrays. One involves the in situ spatially addressable parallel synthesis of oligonucleotide probes by photochemistry [5–7]. The second method involves the direct immobilization of pre-synthesized oligonucleotides [8–10]. In this case, the oligonucleotides are patterned onto a chemically active surface, using spotting or printing technologies, and immobilized through introduction of functional groups on either the 5'- or 3′-oligonucleotide terminus.

Crucial steps in oligonucleotide microarray fabrication are patterning and immobilization of each DNA sequence onto the solid support using appropriate attachment methods. Covalent immobilization methods require chemical modification of the chip surface. Most surface chemistries reported for amino-modified probes are either reactive alkylsilanes on glass supports (e.g. aldehyde- or epoxy-terminated silanes) or thin film polymer coatings bearing reactive groups to amino-modified probes [11–16]. The binding properties of the chemically active surface directly influence the performance of the microarray in terms of signal intensity, signal-to-noise ratio and spot homogeneity by enabling efficient probe-target hybridization. Taking into account these requirements, an ideal support should have an excellent surface chemistry allowing covalent binding of DNA by a stable attachment, a high-binding capacity with an optimal number of reactive sites to which probes can be covalently bonded, and a low background noise. Such constraints lead to a broad variability in the performance of microarrays-based genetic analyses and to substantial increase in the costs associated with microarray-based assay technologies. The preparation of the surfaces and the technology of organosilane coatings required certain precautions. Moreover, this type of process requires a powerful chemical treatment in order to obtain a perfectly clean and homogeneous glass substrate. Thus,

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preparation of such kind of glass functionalization implies many steps which are difficult to control precisely [17,18].

To address some of these challenges, we report the development of a versatile attachment by using the plasma treatment that enables formation of a chemically active surface on glass substrates onto which the DNA probe may covalently bind. Moreover the plasma treatment allows eliminating the different stages of cleaning of the glass substrate as well as the reactions of silanisation usually used for the surface functionalization in such applications. Plasma polymerization takes place when a glow discharge is formed in an organic gas or vapor. The precursor is then fragmented, giving rise to different oligomers that reorganize to form a "plasma polymer". The plasma polymerization of acrylic acid, to produce thin coatings bearing carboxylic functions, has been an active area of research for applications in biomedical and biosensors [19–23].

While plasma polymerization processes enable the deposition of a tremendous variety of functional thin films on any substrate, the application of these deposits can suffer from insufficient adhesion to the substrate or from unpredictable swelling behavior when subjected to aqueous solutions [24]. The majority of the studies that have been performed in 13.56 MHz pulsed or continuous discharges report the fact that their stability is highly affected by washing [25–27]. In a previous work, we used a low frequency discharge of 70 kHz with an asymmetrical configuration of electrodes in order to obtain coatings from acrylic acid precursor [28]. Different physicochemical analyses have been used such as water contact angle measurements, Ellipsometry, Fourier Transform Infrared spectroscopy, Scanning Electron Microscopy and X-ray photoelectron spectroscopy (XPS) analyses to characterize the plasma polymerized acrylic acid (PPAA) coatings deposited under different experimental conditions [28]. Such reactor allows obtaining a coating of PPAA on the surface of polyethylene film, with a strong resistance to washing. In this paper [28], the excited species were analyzed by OES (Optical Emission Spectroscopy) in order to find a correlation between the deposited films and the plasma properties. Indeed, in this paper it has been shown that the higher the dissociation rate of acrylic acid, the higher will be the CO emission, the lower will be the carboxylic functions incorporated in the deposited coatings.

In this study, glass slide has been used as the substrate while in reference [28] polyethylene was the substrate on which PPAA was deposited. Once the plasma parameters were optimized in order to obtain PPAA coatings which were stable to washing on the surface of glass slides, the probe immobilization performance of these microarray surfaces were investigated and compared to the one obtained with commercial slides. For the PPAA surface activation, a simple two-step reaction was reported which allowed to generate the amine-reactive N-hydroxy succinimide (NHS) active groups on that surface. The immobilization efficiencies were quantified by fluorescence intensity analysis after hybridization of complementary labeled targets with three couples of 50-mer DNA printed probes. Microarrays produced from PPAA coatings have shown a high relative density of attached probe DNA molecules and signal-to-background ratio measured for target DNA hybridization. "Specific" versus "nonspecific" probe immobilization efficiencies have been quantified after hybridization of complementary labeled targets with three couples of non-modified and amine-modified DNA printed probes. The data clearly showed that the plasma process gave rise to a strong attachment of DNA onto glass substrates.

2. Experiment

2.1. Materials

Glass substrates were purchased from Gold Seal Products (thickness 0.93–1.05 mm). Oligonucleotides were purchased from Sigma Aldrich Genosys with the following structures (Table 1). Acrylic acid (99% pure), NHS, 1-Ethyl-3-(3-DimethylAminopropyl) Carbodiimide (EDC), and sodium dodecyl sulfate (SDS) were purchased from Sigma Aldrich. 20X saline-sodium citrate (SSC) was provided from Invitrogen. Commercial microarrays slides were purchased from Amersham (Codelink™) and GeneScore [30].

Table 1
Oligonucleotides structures used (A = adenine, C = cytosine, G = guanine, T = thymine).

5′-GTGCCTCACGGTGGTTGCCATCACTGTC-TTCATGTTCGAGTATTTCAGCC-3′	1S
5′-TTTTGAGATCTGGCTTCATTTCGACGCTGAC-GGAAGTGGTTACCTGGAAG-3′	2S
5′-AACGCCCATCTTAAAATCGACGCCT-GTCTCTCCCCCATTGCTCTTACCAG-3′	3S
5′-NH_2-$(CH_2)_6$-GTGCCTCACGGTGGTTGCCATCACT-GTCTTCATGTTCGAGTATTTCAGCC-3′	Amino-1S
5′-NH_2-$(CH_2)_6$-TTTTGAGATCTGGCTTCATTTCGACGC-TGACGGAAGTGGTTACCTGGAAG-3′	Amino-2S
5′-NH_2-$(CH_2)_6$-AACGCCCATCTTAAA-ATCGACGCCTGT-CTCTCCCCCATTGCTCTTACCAG-3′	Amino-3S
5′-(Cy3)-GGCTGAAATACTCGAACATGAAGA-CAGTGATGGCAACCACCGTGAGGCAC-3′	Comp-5′ (Cy3)-1S
5′-(Cy3)-CTTCCAGGTAACCACTTCCGTCAG-CGTCGAAATGAAGCCAGATCTCAAAA-3′	Comp-5′-(Cy3)-2S
5′-(Cy3)-CTGGTAAGAGCAATGGGGGAGAG-ACAGGCGTCGATTTTAAGATGGGCGTT-3′	Comp-5′-(Cy3)-3S
5′-(Cy5)-GGCTGAAATACTCGAACATGAAG-ACAGTGATGGCAACCACCGTGAGGCAC-3′	Comp-5′ (Cy5)-1S
5′-(Cy5)-CTTCCAGGTAACCACTTCCGTCAGC-GTCGAAATGAAGCCAGATCTCAAAA-3′	Comp-5′-(Cy5)-2S
5′-(Cy5)-CTGGTAAGAGCAATGGGGGAGAGACA-GGCGTCGATTTTAAGATGGGCGTT-3′	Comp-5′-(Cy5)-3S

2.2. Plasma reactor

A bell-jar reactor with an asymmetric configuration of electrodes namely a blade-type HV (high voltage) electrode-grounded cylinder was used for the plasma polymerization of acrylic acid. The reactor has been described in detail elsewhere [28]. The stainless steel HV electrode is hollow and serves for the introduction of the gases. It faces a grounded stainless steel cylinder (length 22 cm and diameter 7 cm) which is covered with a PE film. The glass slides were placed on the polymer film with the help of a double adhesive tape. The gaseous mixture was produced by bubbling argon at a rate of 10 sccm in a 10 ml round bottom flask containing the acrylic acid monomer. Under such conditions, the flow rate of acrylic acid was around 6 sccm. The main chamber was evacuated by a TPH 170 (Balzers) turbo molecular pumping system and a base pressure of 10^{-3} Pa was obtained. The operating pressure was maintained at 20–40 Pa during plasma polymerization by an ALCATEL 2012 AC rotary pump resistant to chemicals.

2.3. XPS (X-ray Photo-Electron Spectroscopy)

The XPS spectra were acquired on a Thermo VG ESCALAB 250 spectrometer. The spectra were recorded with a monochromatized Al Kα X-ray source (1486.6 eV, 10 mA, 13 kV) and a combination of electron and argon ion flood guns for charge compensation. The spectra were acquired with pass energies of 150 eV and 20 eV for the survey and the high-resolution spectra, respectively. Quantitative surface analyses were performed using the peak areas of the high-resolution spectra and sensitivity factors provided by the manufacturer. Sample charging was corrected by positioning the hydrocarbon peak component of the C_{1s} signal at 285.0 eV.

2.4. PPAA coating activated by NHS and EDC

The activation of the surfaces has been done in aqueous solution by immersing the slides in 0.025 M NHS and 0.1 M EDC aqueous solutions