



Innovative metal oxide-based substrates for DNA microarrays

A Dante, amico vero

Growing in wisdom while developing knowledge

Lidia Armelao^a, Michele Pascolini^a, Elena Biasiolo^{b,1}, Eugenio Tondello^{b,*}, Gregorio Bottaro^c, Maurizio Dalle Carbonare^d, Antonello D'Arrigo^d, Alberta Leon^d

^a ISTM-CNR and INSTM, Dipartimento di Scienze Chimiche, Università di Padova, Via Marzolo, 1, 35131 Padova, Italy

^b Dipartimento di Scienze Chimiche, Università di Padova and INSTM, Via Marzolo, 1, 35131 Padova, Italy

^c IMIP-CNR and INSTM, Via Orabona, 4, 70126 Bari, Italy

^d Research and Innovation Company, Via Svizzera, 16, 35127 Padova, Italy

ARTICLE INFO

Article history:

Received 30 March 2008

Accepted 31 March 2008

Available online 10 April 2008

Dedicated to Dante Gatteschi.

Keywords:

Sol-gel

DNA microarrays

Iron oxide

Zinc oxide

Functional surfaces

ABSTRACT

In the present report, we propose a novel approach to synthesize DNA microarrays that employs immobilization of the nucleic acid molecules onto zinc and iron oxide surfaces through their phosphate backbone. Oxide films were prepared by the sol-gel technique and the resulting surfaces were characterized especially with respect to surface chemistry and morphological features by both X-ray photoelectron spectroscopy (XPS) and atomic force microscopy (AFM). ZnO films annealed at $T \leq 300$ °C show the most promising surface features to be employed for DNA microarray preparation, *i.e.* high density of binding sites (hydroxyl groups), smooth and homogeneous surfaces, high optical transmittance in the visible spectral range suitable for detection using fluorescence, and easy handling during preparation procedures. The analysis of nucleic acid retention on the oxide layers was performed by the scanning of dye-labelled DNA previously printed on the substrate using the DNA microarray robotic arm. Clearly visible spots with regular shape were revealed above the background noise indicating that anchoring of the DNA on the treated surface is efficient and does not interfere with hybridization processes. The use of suitably engineered zinc oxide film makes the immobilization strategy ideal for facile, efficient, and cost-effective manufacturing of DNA microarrays.

© 2008 Published by Elsevier B.V.

1. Introduction

It is expected that biomedical nanotechnology will soon produce major advances in molecular diagnostics, therapeutics, molecular biology and bioengineering. In diagnostic assays, biomaterial devices and surface-related bioanalytical applications, the controlled immobilization of biomolecules onto surfaces is an important aspect for monitoring the specific interactions and binding of biomolecules or cells [1]. Among diagnostic devices, DNA microarrays are important biomedical research tools owing their power and versatility to the ability to simultaneously sequence and specifically capture target DNA onto thousands of probe spots. DNA microarrays consist of a flat substrate grafted with various known DNA probes at predefined locations [2]. They are usually fabricated by the photolithographic on-chip synthesis of oligonucleotides or by spotting DNA or oligonucleotides droplets onto so-

lid supports. Irrespective of the chosen method of microarray fabrication, the surface of the solid substrates plays an essential part in the use and performance of DNA microarrays [1,3]. To a large extent selectivity, sensitivity and reproducibility of a DNA sensor are determined by the surface chemistry of the DNA recognition interface. Among the adopted substrates, plastic and inorganic materials like silicon wafer, gold or silicon nitride [4–17] have been reported. The substrate most widely used for the fabrication of microarrays is silica or glass, and several surface modifications are available for the adsorption and covalent immobilization of DNA. For covalent tethering, the silanol groups of the substrate are usually first reacted with alkoxy-silanes such as aminopropyl triethoxysilane or glycidoxypentyl trimethoxysilane to form a silane layer displaying amino, epoxide, or aldehyde groups [10–16]. Chemically modified DNA is then commonly coupled to the surface via ester, ether, or imine linkages, or in the case of heterobifunctional cross-linkers via a thioether linkage. Despite its popularity, the conventional silanization of silica substrates remains challenging as the modification tends to produce irreproducibility in the macroscopic properties (*e.g.*, wettability and contact angle hysteresis) of the deposited monolayers [3,18]. The

* Corresponding author.

E-mail address: eugenio.tondello@unipd.it (E. Tondello).

¹ Present address: Matech, Parco Scientifico e Tecnologico Galileo, Corso Stati Uniti 14, 35127 Padova, Italy.

molecular reason for the problems is thought to originate from the complex chemistry of the multistep silanization reaction leading eventually to the formation of three-dimensional aggregates or multilayers instead of a pure monolayer [19–21].

In this report, we explore alternative surfaces – nanostructured metal oxides layers – as innovative substrates for DNA microarrays. Among the inorganic materials, metal oxides play a fundamental role as they present a considerable variety of structures, stoichiometries and chemico-physical properties which can be tailored exploiting suitable and versatile synthesis techniques. This variety of optical, catalytic, magnetic and electrical properties [22] discloses interesting perspectives for the design and the preparation of innovative functional systems, thanks to the possibility of controlling their organization on the *nano*-dimensional scale [23]. In fact, this size domain implies a much larger surface-to-volume ratio, together with an enhanced or different chemical reactivity, with respect to conventional materials [24,25]. In this view, nanotechnologies offer to biology new extremely interesting tools [26] paving the way for the development of next generation DNA chip. In order to be employed in the microarray fabrication, a solid support must satisfy two key requirements, *i.e.* high binding capability with DNA molecules and suitability for high-throughput manufacturing and screening procedure [27] on which both reproducibility and reliability of the final results strongly depend [28]. Among the available sites, DNA molecules are expected to directly link at the oxides surface through the phosphate backbone. With this aim in mind, we decided to study zinc and iron oxides as DNA binding surfaces due to the well-known strong chemical affinity between Zn(II) and Fe(III) ions towards phosphate groups and due to their biocompatibility that cannot be disregarded in this kind of applications. In the last decade, our research team has developed a significant experience in the synthesis of metal–oxide thin films and nanosystems, and in particular in the sol–gel preparation of zinc and iron oxides [29–35]. Sol–gel represents a versatile low-temperature chemical approach (*soft chemistry*) for producing thin films with good control on morphology and microstructure. Such systems have numerous advantages, which include the ease of surface preparation, an excellent stability and low production price with limiting amounts of steps.

In this work, ZnO and Fe₂O₃ thin films have been prepared as DNA microarray substrates by sol–gel dip-coating starting from ethanolic solutions of zinc acetate and iron nitrate [29–35], respectively. The obtained layers, after annealing in air, have been employed as surfaces for DNA grafting through a standard spotting procedure. The obtained results are presented playing particular attention to the interplay between the performance of the obtained DNA microarrays and the chemical and microstructural surface characteristics of the metal oxides thin films.

2. Experimental

2.1. Synthesis

Zinc acetate dihydrate (Zn(OOCCH₃)₂ · 2H₂O, Fluka 99%) was adopted as a source compound for ZnO. The precursor was used as received without any further purification. ZnO films were prepared starting from ethanolic solutions of the precursor compound (C_{ZnO} = 30 g/l). The sol–gel reactions occurred under acid conditions by adding water and acetic acid to the precursor solutions (H₂O/Zn = 11, CH₃COOH/Zn = 1) [29–31]. The solutions were stirred at 65 °C for 2 h before film deposition by dip-coating at room temperature. Depositions were performed on suitably cleaned Heraeus® silica slides (Heraeus®, Quarzschmelze, Hanau, Germany)

2.5 × 7.5 × 1 mm each, with a withdrawal speed of 10 cm min^{−1}. The obtained layers were annealed between 100 and 500 °C for increasing times up to 2 h, resulting homogeneous, colourless and well adherent to the substrate. All handling operations and thermal treatments were performed in air.

Iron nitrate (Fe(NO₃)₃ · 9H₂O, Aldrich 99%) was chosen as a precursor compound for iron oxide films that were deposited from the corresponding alcoholic solutions (C_{Fe₂O₃} = 17 g/l, pH < 3) [32–35]. Dip-coating depositions and annealing were performed in the same conditions adopted for zinc oxide films yielding orange-brown, smooth and crack-free Fe₂O₃ layers.

2.2. Film characterization

The surface chemical composition was analyzed by X-ray photoelectron spectroscopy (XPS) measurements performed on a Perkin–Elmer Φ 5600ci spectrometer using a monochromatized Al Kα radiation (1486.6 eV), at a working pressure lower than 10^{−9} mbar. The specimens, mounted on steel sample holders, were introduced directly into the XPS analytical chamber by a fast entry lock system. The sample analysis area was 800 μm in diameter. Survey scans were run in the 0–1300 eV range. Detailed spectra were recorded for the following regions: Zn2p_{3/2}, Zn3p, ZnLMM, Fe2p, O1s and C1s. The reported Binding Energies (BEs, standard deviation = ±0.2 eV) were corrected for charging effects assigning to the adventitious C1s line a BE of 284.8 eV [36]. The analysis involved Shirley-type background [37] subtraction, and, whenever necessary, spectral deconvolution which was carried out by non-linear least-squares curve fitting, adopting a Gaussian–Lorentzian sum function. The atomic composition of the samples was calculated by peak integration, using sensitivity factors provided by the spectrometer manufacturer (Φ V5.4A software) and taking into account the geometric configuration of the apparatus.

Optical absorption spectra of the films were recorded in the range 300–800 nm on a Cary 5E (Varian) UV–Vis–NIR dual-beam spectrophotometer with a spectral bandwidth of 2 nm. In each spectrum, the silica substrate contribution was subtracted.

Glancing incidence X-ray diffraction (GIXRD) measurements were performed by a D8 Advance Bruker diffractometer equipped with a Cu Kα source (40 kV, 40 mA) and a Göbel mirror, in the 20–55° 2θ range at a fixed incidence angle of 0.5°.

Sample morphology was investigated by AFM (atomic force microscopy). Micrographs were recorded by a Autoprobe CP instrument (Park Scientific) operating in contact mode and in air. Images were collected on different sample areas in order to check surface homogeneity.

2.3. Spotting and DNA immobilization

For DNA deposition, a 12-pin Gen III spotter was utilized (Amersham-Pharmacia, Little Chalfont, UK). Spotting volume was about 1 nl and DNA concentration ranged between 200 and 400 fmoles/μl in 50% DMSO aqueous solutions. Depositions were performed at 20 °C and 55% relative humidity. After spotting, the slides were exposed to a 20 s UV-C pulse at 65 mJ/cm² in a Hoefer UVC 500 UV cross-linker (Hoefer Inc. San Francisco CA).

Total RNA was extracted from human-derived cell cultures (SK-N-BE) with Trizol (Invitrogen Milano, Italy). Ten micrograms of total RNA was used in each labelling reaction. Labelling and hybridization of the slides were conducted with the Array50 system (Genisphere Inc., Hatfield, PA), according to the manufacturer's instructions. Hybridized cDNAs were fluorescently labelled with both Cy3 (λ_{em} = 570 nm) and Cy5 (λ_{em} = 670 nm) cyanines. Spot visualization was achieved by laser scanning of the fluorescent slides by a Gen III scanner (Amersham/Little Chalfont, UK). The

Download English Version:

<https://daneshyari.com/en/article/1311542>

Download Persian Version:

<https://daneshyari.com/article/1311542>

[Daneshyari.com](https://daneshyari.com)