Sensors and Actuators B 231 (2016) 412–422

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

Novel polymeric coatings with tailored hydrophobicity to control spot size and morphology in DNA microarray

Laura Sola*, Francesco Damin, Marina Cretich, Marcella Chiari

Istituto di Chimica del Riconoscimento Molecolare, CNR, Via Mario Bianco, 9, 20131 Milano, Italy

ARTICLE INFO

Article history:
Received 3 November 2015
Received in revised form 25 January 2016
Accepted 12 March 2016
Available online 15 March 2016

Keywords:
Microarrays
Polymeric coating
Surface modification
Spot morphology
Hydrophobic/hydrophilic surface

ABSTRACT

The fabrication of high density microarrays requires a precise control of surface physical and chemical properties. In fact, spot morphology has a deep impact on signal level and might affect seriously the reliability and reproducibility of the assay. As a consequence, a proper biomolecule deposition and immobilization increases the accuracy of data analysis. Here we propose a new polymeric coating with intermediate hydrophilic/hydrophobic characteristics able to control the spreading of spots onto the surface as well as their size, thus enabling the reduction of the pitch between spots. The new coating exemplifies the versatility of a copolymer system based on the simultaneous presence of *N*,*N*-Dimethylacrylamide (DMA), 3-(trimethoxylsilyl)propyl methacrylate (MAPS) and fluorinated monomers, which impart to the surface a more hydrophobic behavior, reducing the drops spreading and merging. The controlled hydrophobicity allows to control also the size of the spots, thus the possibility of reducing their pitch to 140 μm and to obtain arrays with smaller dimentions, a very useful characteristics in those applications which imply the use of reduced areas, for example in miniaturized biosensors or microfluidic devices. The obtained copolymer is easily adsorbed onto glass or silicon oxide from a diluted aqueous solution of the polymer and the result is a microarray surface with intermediate hydrophobic/hydrophilic properties (average water contact angle is 50° and surface energy values are reduced from 114 mN/m to 50 mN/m), which allows decrease of the spot distance without merging of the droplets. To further improve the spot size and morphology, we have also investigated the use of several detergents added to the spotting buffer: in particular, the use of sucrose monolaurate, together with the hydrophobic surface, permitted the fabrication of surfaces for dense DNA microarray with very high fluorescence signals and low background noise. In fact, as an example of application in DNA microarray, the fluorinated coating has been used for the genotyping of KRAS G12D mutation, a common variant in the KRAS gene implicated in the colorectal cancer.

1. Introduction

In recent years, the microarray technology has emerged as an important technique with wide applications in different areas of biomedical analysis thanks to its versatility and miniaturization capability. In order to accomplish a large number of genomic or proteomic tests in parallel, a microarray substrate is functionalized with thousands of probes bound to a solid surface in pico or femto moles (10^{-12},10^{-15} mol) amounts. As a consequence, the physical and chemical characteristics of the interfacial layer between the biomolecules and the inorganic substrate are key factors for the success of the technique. In fact, proper biomolecule deposition and immobilization are crucial to increase the accuracy of data analysis as spot morphology has a great impact on the signal level: non homogeneous spots, such as doughnut-shape spots or coffee ring effect may cause errors in the acquisition and measurement of signals, thus affecting the reliability of the assay [1]. Furthermore, advances on micro fabrication techniques have led to the development of miniaturized and fully integrated solid phase analytical devices that imply a scaling down of the entire analytical system and process while maintaining high sensitivity. The miniaturization of the system requires a perfect control of the surface properties in order to maximize probe immobilization in reduced areas, avoiding spot merging and cross contamination.

Several strategies have been adopted to control spot morphology and merging. In particular, considering the drying rate of a diluted droplet placed on a surface, hybrid hydrophobic/hydrophilic substrates have been introduced: Moran-Mirabal

* Corresponding author.
E-mail address: laura.sola@icrm.cnr.it (L. Sola).

http://dx.doi.org/10.1016/j.snb.2016.03.049
0925-4005/

and collaborators have solved the problem of coffee ring effect by fabricating a polymer liftoff surface which combines a hydrophobic polymer film of Parylene with photolitographically patterned hydrophilic areas of a Self Assembled Monolayer (SAM), where the DNA is deposited. The part of the drop in contact with the hydrophobic area undergoes, through evaporation, an inward capillary flow which reduces the coffee stain effect [2]. Similarly, Lee and collaborators introduced a hybrid substrate constituted of two different types of SAMs one hydrophobic and one hydrophilic, obtained, again, through photolithography [3]. Even though these approaches solve the issue of irregular spots and cross-contamination, facilitating the assay analysis, they are very complicated to perform and involve the use of SAMs which show several drawbacks such as thermal instability, limited range of functional groups displayed on the surface, long reaction times to obtain monolayers [4]; furthermore, the two-dimentional arrangement of SAM restricts the maximal surface density of the functional moieties and provides limited accessibility of functionalities [5]. Three-dimentional surfaces, instead, provide a homogenous surface presenting high concentration of reactive groups, resulting in an increased binding capacity of targets compared to monodimentional films [5]. Ultimately, they act as linkers distributing the bound probe also in the axial position, thus causing a faster reaction with the target involved in biomolecular recognition. Recently, a 3D-hydrogel was presented as a method to immobilize DNA, in order to increase probe density [6]. The hydrogel is photopolymerized onto a particular support, called PolyShrink, a thermosensitive material, which offers the possibility of reducing the array dimentions by heating the surface. As a consequence, also the height of the spot increases (up to 6 μm), promoting hybridization, but the immobilization procedure is quite complicated as it consists in the deposition of a mixture of a pre-synthesized polymer together with the oligonucleotides, which are then photopolymerized; the entire array is then cured at 160 °C to shrink the surface, a temperature which is not compatible with several biological systems. In 2004, Pirri and collaborators [7] proposed a polymer coating realized by a combination of physisorption and chemisorption that promotes the attachment of biomolecules by exposing functionalities such as active esters reactive towards the nucleophile groups of proteins, peptides or amino-modified DNA. In particular, this copolymer, poly(DMA-NAS-MAPS), is constituted of three monomers: *N, N*-dimethylacrylamide (DMA) that binds to the surface by weak non-covalent interactions such as hydrogen bonding, Van der Waals or hydrophobic forces, *N*-acryloyloxysuccinimmide (NAS) a chemically reactive monomer that covalently binds DNA and proteins to the surface, and a pending silane hydrolyzable monomer, 3-(trimethoxysilyl) propyl methacrylate (MAPS), which promotes the condensation of the polymer with surface silanols. The coating is obtained by simply immersing the support (glass [7], silicon oxide [8,9], nitrocellulose [10], gold [11], plastics [12]) in a diluted aqueous solution of the polymer. Beside the semplicity of its use, another peculiar characteristic of this coating is its hydrophilicity, which results in high resistance to nonspecific binding and low background signal [12]. Unfortunately, the hydrophilicity of a surface has important consequences on the process of deposition of liquid droplets. When the distance between spots must be kept low to increase spot density, it is important to control how the liquid spreads out over the surface as well as the size of the spot. This latter parameter is related to the hydrophobicity of the surface as measured by the contact angle. A low contact angle (<45°) indicates a hydrophilic surface with good wetting properties on which water readily spreads and sticks. A high contact angle (>90°) denotes a hydrophobic surface where water does not interact forming droplets that do not adhere to the surface but are easily displaced. A desirable coating must prevent excessive spreading of the droplets to concentrate the probe in small areas. However, a firm attachment to the underlying surface is also needed so that the droplets remain in position on the surface and dry-out to produce a spot with a reproducible size and uniform intensity. In some circumstances, the poly(DMA-NAS-MAPS) coating provides a surface which is too hydrophilic to allow the creation of an array of small sizes with minimized spot-to-spot distance: reduced spotting areas are, in fact, typical in miniaturized biosensors, microfluidic devices and LoC.

To overcome this problem, we propose a new coating that exemplifies the versatility of a copolymer system based on DMA and MAPS monomers. It shows how easy is to modulate the properties of the surface by simply modifying a minor component of the copolymer.

Here we introduce a new copolymer which provides all the advantages of poly(DMA-NAS-MAPS) in terms of simplicity of coating production, high probe binding density, tri-dimensionality and low background signal, but, thanks to the presence of an additional hydrophobic monomer, forms a more hydrophobic coating able to control how the liquid spreads out over the surface as well as the size of the spot, thus the possibility of reducing the pitch between spots. We have explored the use of several fluorinated monomers, as minor component of the system, which impart to the surface low energy: they include, among others, 1H,1H-perfluoroheptyl acrylate (PFHA), 1H,1H,2H,2H-perfluorodecyl acrylate (PFDA), 1H,1H,2H,2H perfluorododecyl acrylate (PFDDA). The coating process is another main advantage of the copolymers as they can be easily adsorbed onto glass or silicon oxide by simply immersing the substrates into a diluted aqueous solution of the polymer: the result is a microarray surface with intermediate hydrophobic/hydrophilic properties, which also allows a decrease of spot-to-spot distance without merging of the droplets, a very useful characteristic in those applications which imply the use of reduced areas, for example in miniaturized biosensors or microfluidic devices.

To further improve the spot morphology, we have also investigated the use of several detergents added to the spotting buffer: surfactants, in fact, are widely used as additives in spotting buffers as they decrease surface tension and change the evaporation rate of the drops onto the surface, thus improving the spot morphology, the hybridization process and the overall assay performance.

The fluorinated coating has been used for the genotyping of KRAS G12D mutation, a common variant in the KRAS gene implicated in the colorectal cancer, as an example of application in DNA microarray.

2. Materials and methods

N,N-Dimethylacrylamide (DMA), 3-(trimethoxyylsilyl) propyl methacrylate (MAPS), 1H,1H-perfluoroheptyl acrylate (PFHA), 1H,1H,2H,2H-perfluorodecyl acrylate (PFDA), 1H,1H,2H,2H perfluoro dodecyl acrylate (PFDDA), α,α′-Azoisobutyronitrile (AIBN), anhydrous tetrahydrofuran (THF), ammonium sulphate ($(NH_4)_2SO_4$), phosphate buffered saline (PBS), ethanol (EtOH), sucrose monolaurate, Brij 30% solution in water, betaine, sulfobetaine, Tween 20, Triton X100 ethanolamine were purchased from Sigma Aldrich (St. Louis, MO, USA). All solvents were used as received. *N*-acryloyloxysuccinimide was synthesized as reported elsewhere [13]. Oligonucleotides for hybridization testing were synthesized by MWG-Biotech AG (Ebevsberg, Germany) and contained the following sequences: COCU8: 5′-NH2-GCCCACCTATAAGGTAAAAGTGA-3′, COCU10: 5′-Cy3-TCACTTTTACCTTATAGGTGGGC-3′. COCU10 was labeled with the fluorophore Cyanine 3. These oligonucleotides were freeze-dried and re-suspended in DI water at a final concentration of 100 μM before use. Untreated silicon slides 1000 Å Thermal Oxide (14 × 14 mm) were supplied by SVM, Silicon Valley Microelectron-